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α-Bisabolol, a possible safe penetration enhancer for dermal and transdermal therapeutics

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Summary

α-Bisabolol, an inflammatory-inhibiting sesquiterpene, was assessed for its ability to enhance transepidermal drug penetration, in vitro. Human skin samples pretreated with a 1 : 1 α-bisabolol-propylene glycol mixture were 17-fold more permeable to 5-fluorouracil (5-FU) and 73-fold more permeable to triamcinolone acetonide (TACA) with respect to untreated skin. Differential scanning calorimetry of treated stratum corneum samples showed a dramatic decrease in the lipid transition enthalpy, suggesting increased lipid fluidity. Determinations of drug distribution indicated that the stratum corneum-vehicle partition coefficient of 5-FU was unaffected by the enhancer. The solubility ratio of TACA between the enhancer and the vehicle was very low in comparison with the increase in skin permeability. Hence, for both drugs the enhanced penetration in the presence of α-bisabolol arose predominantly from an increase in their diffusivities across the modified skin barrier.

Introduction

In recent years, considerable progress has been made in skin and transdermal medication, yet there are still many problems to overcome. Amongst other difficulties the relative impermeability of the intact stratum corneum and its biological variability pose challenges to investigators. In previous years considerable research has been performed with powerful solvents as possible penetration enhancers, such as dimethyl formamide, dimethylacetamide (Munro and Stoughton, 1965), and dimethyl sulphoxide (Chandrasekaran et al., 1977). As a result of the growing demand for safe materials capable of reversibly reducing the skin barrier resistance to drug permeation, the variety of compounds investigated has been considerably extended, e.g. pyrrolidones (Southwell and Barry, 1983), azocycloheptan-2-one (Stoughton and McClure, 1983) and its derivatives (Bouwstra et al., 1989), *cis*-unsaturated fatty acids and alcohols (Cooper, 1984), cyclohexanone derivatives (Nagai et al., 1988), ethanol (Ghanem et al., 1987) and urea analogues (Williams and Barry, 1989a). Recently, some terpenes have been re-

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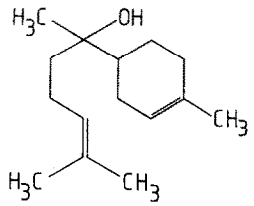

Fig. 1. The structural formula of α-bisabolol: 1-methyl-4-(1,5-dimethyl-1-hydroxyhex-4(5)-enyl)cyclohexen-1.

ported to increase transdermal drug penetration (Zupan, 1983; Nuwayser, 1988; Williams and Barry, 1989b). Among a wide variety of naturally-occurring terpenoids, oxide terpenes such as ascaridole, 7-oxabicycloheptane and 1,8-cineole were found especially effective in decreasing human skin resistance to drug penetration (Barry and Williams, 1989; Williams and Barry, 1989c, 1990) and we have studied such compounds further.

α-Bisabolol is a monocyclic unsaturated tertiary sesquiterpene: 1-methyl-4-(1,5-dimethyl-1-hydroxyhex-4(5)-enyl)cyclohexen-1 (Fig. 1). It is either extracted from natural raw materials (the essential oil of camomile may contain up to 50% (−)-α-bisabolol) or it can be manufactured synthetically as (±)-α-bisabolol. It has an oral (rat) LD_{50} greater than 5 g/kg with zero primary skin irritation (rabbit, BASF test). α-Bisabolol has been proved, by several methods, to have an anti-inflammatory effect as well as preventing skin irritation and reducing the duration of the healing phase. The optically active terpene (−) was identified as the pharmacologically active compound (BASF and Dragoco Active Substances Catalogues). It is used as an inflammatory-inhibiting agent in high quality skin and mouth care products. Being the main counter-irritant ingredient of camomile it also serves to standardize camomile extracts in pharmaceutical preparations, e.g. gargles, drops, salves, etc.

In the view of the reported enhancing properties of some terpenoids and the appropriate pharmacological and physico-chemical properties of α-bisabolol (safe, stable, colourless, non-staining, faint pleasant odour), the present study aimed to investigate the effects of this substance on the transdermal flux of model drug compounds, 5-fluorouracil and triamcinolone acetonide, and on some physical properties of the skin barrier. The skin-compatible bisabolol was formulated in propylene glycol, a synergistic solvent for terpenes (Barry and Williams, 1989).

Materials and Methods

Chemicals

All chemicals used were of the highest purity commercially available. 5-[6-^{3}H]Fluorouracil (5-FU) was obtained from New Research Products, unlabelled 5-fluorouracil from Sigma Chemical Co. and [1,2,4(n)-^{3}H]triamcinolone acetonide (TACA) was obtained from Amersham International. (±)-α-Bisabolol (BIS) was obtained from BASF, and propylene glycol (PG) was from BDH.

Skin preparation

Abdominal human cadaver skin obtained at post-mortem and stored at −24°C was immersed for 45 s in water at 60°C and the epidermis was eased gently away from the underlying dermis using the tip of a gloved finger.

Preparation of radiolabelled saturated donor solution

Excess of solute was added to the appropriate vehicle (water or 1:1 propylene glycol/water mixture) in vials. The sealed vials were shaken for 48 h (for 5-FU) or 1 week (for TACA) at 32 ± 1°C, in a thermostatted water bath. The radiolabelled drug (150 μCi/ml) was added to the supernatant.

Diffusion experiments

An automated diffusion apparatus was used at 32 ± 1°C, (Akhter et al., 1984). The hydrated epidermis was cut into pieces (approx. 1 × 1 cm) and mounted in stainless steel diffusion cells where the skin area interposed between the donor and the acceptor was 0.126 cm^2 (0.4 cm in diameter). The acceptor consisted of 0.002% aqueous sodium azide solution, degassed with helium, perfusing through the acceptor compartment at a flow rate of 2 ml/h, a rate which ensured sink conditions. After the membranes had equilibrated, 150 μl of penetration enhancer (PG, BIS or 1:1 PG/BIS

mixture) was pipetted onto the skin and left overnight.

The donor compartments of the cells containing penetration enhancer were then rinsed several times with distilled water and the skin surface was blotted gently with a tissue. The acceptor perfusate was collected for 2 or 3 h (in order to determine the blank values) and 150 μl of a saturated donor solution of drug was pipetted onto the skin and the donor compartments were sealed. Perfusate samples (4 and 6 ml for 5-FU and TACA, respectively) were collected periodically, mixed with 14 ml of a liquid scintillant (Optiphase 'Hisafe II', Pharmacia) and their radioactivities were measured.

The steady-state flux of each drug was determined from the linear regions of the cumulative penetration curves. The permeability coefficient (K_p) was calculated from the steady-state flux and the drug solubility values at 32°C.

Determination of drug solubility at saturation

For TACA, 1 ml of the supernatant (filtered through a 0.2 μm Millipore membrane) was diluted (×100 or 1000) in 4:6 acetonitrile-water mixture; 25 μl samples were directly injected into a reverse-phase HPLC column (C18, 5 μm, 10 cm × 5 mm) with the detector set at 240 nm. The mobile phase consisted of acetonitrile : water 4 : 6 at a flow rate of 0.4 ml/min. Under these conditions the retention time of TACA was 1.4 minutes.

The aqueous saturation solubility of 5-FU is 10.2 mg/ml (Bond and Barry, 1988).

Determination of stratum corneum-water partition coefficient of 5-FU

The method of Barry and Bennett (1987) was employed using human stratum corneum from four different sources. Weighed discs (2.5 cm diameter) of dried stratum corneum were placed in vials containing 1 ml of radiolabelled saturated 5-FU aqueous solution and shaken in a thermostatted water bath at 32 ± 0.5°C for 48 h. The samples were rinsed in distilled water, blotted on a filter paper, weighed and dissolved in 2 ml of a tissue solubilizer (Soluene-350) in glass scintillation vials. 14 ml of liquid scintillation fluid and 140 μl of glacial acetic acid were added to each vial and the samples were stored at room temperature overnight to allow chemiluminescence to subside. The radioactivity was analysed with respect to blanks and standards prepared in vials containing dissolved stratum corneum disks. The radioactivity in 100 μl of each aqueous vehicle was also determined.

Differential scanning calorimetry

Stratum corneum was obtained by treating epidermal samples with 0.002% trypsin solution containing 0.5% sodium bicarbonate at 37°C overnight. The stratum corneum was dried for 48 h over silica in a vacuum desiccator, weighed and rehydrated over saturated potassium sulphate solution for 48 h to about 50% water content. Treatments with PG or 1 : 1 BIS : PG were performed by soaking the hydrated stratum corneum samples in the appropriate substance overnight. The treated skin samples were blotted on a filter paper prior to the thermal analysis. A Perkin-Elmer DSC 7 Series Thermal Analysis System was used; data were derived using hermetically-sealed stainless steel pans at a heating rate of 10°C/min over a temperature range of 10–130°C (Goodman and Barry, 1989).

Results and Discussion

Fig. 2 demonstrates the effects of pretreatments with PG, BIS and 1 : 1 BIS : PG on the penetration of the polar compound 5-FU, delivered from its saturated aqueous solution. The corresponding fluxes and permeability coefficients (K_p) values are listed in Table 1. Whereas PG exhibited a relatively small effect on the drug penetration, 5.4- and 16.9-fold increases in penetration were observed following pretreatments with BIS and BIS/PG, respectively.

Fig. 3 shows the flux profile of 5-FU through BIS- and BIS/PG-treated skin samples as a function of time. The high drug flux through the PG/BIS-treated skin was characterized by a short lag phase. However, after about 10 h, the flux gradually declined. The difference between the fluxes (through BIS/PG- and BIS-treated skin samples) diminished progressively and by the end

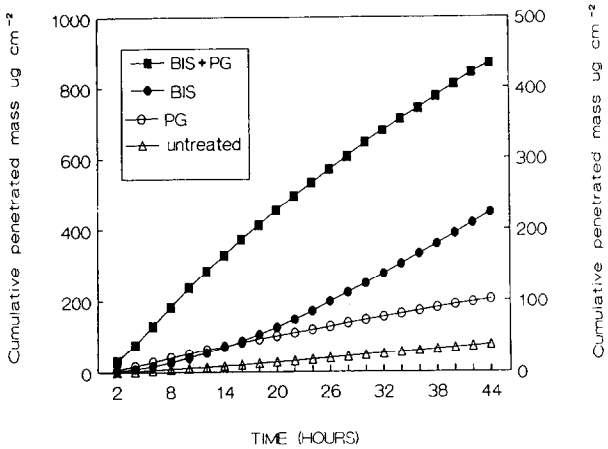

Fig. 2. Example cumulative penetration curves of 5-fluorouracil delivered from its saturated aqueous solution to untreated and enhancer treated human epidermis at 32°C. The left ordinate refers to the α-bisabolol/propylene glycol-treated skin and the right ordinate refers to all other curves.

TABLE 1

Penetration parameters of 5-fluorouracil delivered to untreated and treated human epidermis from saturated aqueous solution at 32°C

Pre-treatment	N [a]	Flux ($\mu g\ cm^{-2}\ h^{-1}$) ($\pm$SE)	K_p ($cm\ h^{-1}$) ($\times 10^4$)	K_m [b]	D [c] ($cm^2\ h^{-1}$) ($\times 10^6$)
Untreated	5	1.23 ± 0.18	1.20	0.81	0.5
PG	2	2.14 ± 0.12	2.20	–	–
BIS	4	6.58 ± 0.94	6.42	0.90	2.5
1:1 BIS/PG	5	20.8 ± 0.90	20.3	–	–

[a] Number of runs.
[b] The experimental stratum corneum-water partition coefficient (see Table 2).
[c] The diffusion coefficient calculated from $D = K_p h / K_m$ where K_p is the experimental permeability coefficient and h is the barrier thickness (35 μm used).

of the experiment (time 43 h), it was less than 2-fold. In the view of the synergistic effect achieved by using some penetration enhancers in conjunction with PG (Cooper, 1984; Barry, 1987; Barry and Williams, 1989), it seems that this flux decline may arise from continual washout of PG from the skin, so decreasing the synergistic effect. Indeed, no decline was observed in TACA penetration through BIS/PG-treated skin when PG was continuously supplied from the vehicle.

In order to determine whether a shift of the partition coefficient contributed to the enhance-

TABLE 2

Determination of the stratum corneum-water partition coefficient (K_m) of 5-fluorouracil at 32°C for untreated and α-bisabolol-treated skin

Skin code	Dry weight (mg)	Wet weight (mg)	mg drug/g s.c. (wet)	mg drug/g water	K_m	Average K_m ($\pm$SD)
Untreated skin						
1	3.51	14.44	6.28	10.13	0.62	
2	4.41	18.10	7.55	10.20	0.74	
2	4.26	17.48	7.03	10.04	0.70	0.81 ± 0.15
3	2.55	7.74	9.58	7.79	0.98	
3	2.54	8.35	10.3	9.90	1.04	
4	6.27	21.8	8.15	10.17	0.80	
4	6.71	33.7	7.55	9.93	0.76	
Treated skin						
1	3.20	10.45	7.93	10.17	0.78	
1	4.58	13.77	7.56	9.95	0.75	
2	3.47	11.65	6.65	10.34	0.77	
2	4.70	14.76	8.62	9.68	0.89	0.90 ± 0.17
3	2.53	7.15	10.38	10.08	1.03	
3	2.07	5.27	12.43	9.95	1.25	
4	6.17	19.54	8.21	9.89	0.83	
4	7.49	19.68	8.76	9.84	0.89	

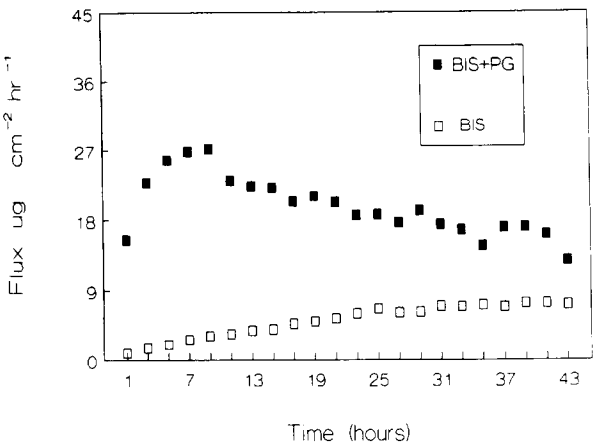

Fig. 3. The flux of 5-fluorouracil through α-bisabolol and α-bisabolol/propylene glycol-treated human epidermis at 32°C (example plots).

ment effect, the stratum corneum-water distribution of 5-FU was determined (Table 2). Partition coefficient values of 0.81 ± 0.15 and 0.90 ± 0.17 for the untreated and BIS-treated skin, respectively, indicated that little effect on the drug partitioning was involved.

Knowing the partition coefficient and the permeability coefficient, the apparent diffusion coefficients (D) were calculated for the treated and the untreated skins using a nominal 35 μm skin thickness and ignoring tortuosity effects (see Table 1). The D values were in a ratio of 5:1, BIS-treated vs untreated, suggesting a dominant effect of the enhancers on diffusional processes.

During the procedure of the drug partitioning determination, the skin samples were analysed for water uptake (all samples) and BIS uptake (treated samples). It was found (Table 3) that BIS did not increase the water uptake of the skin; in fact, it caused a significant decrease. This observation may be of some relevance when one tries to suggest a possible mechanism for the enhancement effect since water may increase fluidity of stratum corneum lipids and facilitate the mobility of hydrophilic and lipophilic drug molecules (Barry, 1987).

In order to explore a possible effect of this enhancer on the skin lipids, differential scanning calorimetry (DSC) was employed. In a typical hydrated sample of human stratum corneum, four main endothermic transitions are observed across the temperature range: endotherm 1 which is ascribed mainly to the melting of surface lipids; endotherm 2 which depends on melting of lipid chains in the bilayer structure; endotherm 3 which is ascribed to break up of lipid-protein associations, or of polar head group associations and disruption of cholesterol-stiffened regions; and endotherm 4, the intracellular keratin peak (Barry, 1987; for a review of this controversial field, see Goodman and Barry, 1989).

The thermograms obtained for untreated and treated skin samples were analysed and the relative values of the total energy involved in the two main endothermic lipid transitions (endotherms 2 and 3) are listed in Table 4. Untreated skin showed two lipid transition peaks, one near 75°C and the other near 88°C. BIS/PG-treated skin provided only one lipid transition peak near 71°C, the total lipid transition energy decreasing dramatically with respect to the control. PG decreased the peak temperatures of both transitions but did not affect enthalpy values. These DSC results are very similar to those reported recently for Azone derivatives in PG (Bouwstra et al., 1989); PG did not

TABLE 3

Water and α-bisabolol uptake (mg per mg dry tissue) of untreated and bisabolol-treated skin samples

Skin samples	Water uptake [a] (±SE)	Bisabolol uptake [a] (±SE)
Untreated	2.83 ± 0.22	not applicable
Treated	1.69 ± 0.11	0.30 ± 0.04

[a] $n = 8$, t-test level of significance, $p < 0.001$.

TABLE 4

Effects of α-bisabolol and propylene glycol on the total enthalpy change involved in the lipid phase transitions (endotherms 2 and 3): the values are normalized with respect to those of the untreated skin (4.7 and 4.0 J/g for skin A and B, respectively)

Pretreatment	Skin A	Skin B
PG	1.12	0.99
1:1 PG/BIS	0.10	0.16
BIS	0.08	0.25

decrease (in fact, it slightly increased) the enthalpy involved in the lipid transitions while Azone derivatives in PG showed only one lipid peak with a considerably lower ΔH. Goodman and Barry (1989) found that upon treating samples of hydrated human stratum corneum with Azone in PG, the lipid transitions (endotherms 2 and 3) became smaller as Azone concentration rose. These two transitions were much reduced (however, still present) after the 5% Azone treatment. In the present study it was also found that in the pure state (without PG) BIS had a considerable effect on the lipid transitions as shown in Table 4 (although there were considerable differences between the skin samples tested). Nevertheless, BIS alone was much less effective as a penetration enhancer than when combined with PG as assessed in permeation studies. This finding may imply that a compound which shows an effect on the stratum corneum thermogram is not necessarily a good penetration enhancer. Furthermore, in agreement with the results reported by Goodman and Barry (1989) for PG/Azone-treated stratum corneum and in contrast to the results obtained by Bouwstra and coworkers (1989) for PG/Azone derivatives, the protein denaturation peak (T4) did not disappear in BIS/PG-treated skin or in PG-treated skin.

Taking the following facts under consideration: (a) treating the skin with BIS does not significantly affect the stratum corneum-water partitioning of 5-FU, (b) BIS exhibits a dramatic effect on the skin lipids transition enthalpy, and (c) the enhancer decreases lag times, we conclude that the enhancement effect arises predominantly from BIS increasing the diffusion coefficient of 5-FU across the skin barrier, i.e. by disrupting the highly ordered lipid structure and by thus increasing permeant mobility.

The study was extended to determine any enhancing effect of BIS on the transdermal penetration of a more lipophilic drug, TACA. Because of the very poor water solubility of this drug it was decided to use a saturated 1 : 1 PG : water solution as the donor solution. Fig. 4 demonstrates the impressive effect on TACA penetration following pretreatment of the skin with BIS/PG. The corresponding flux and K_p values are listed in Table 5.

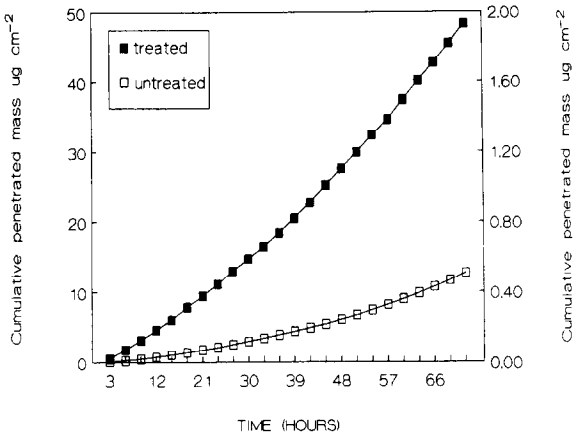

Fig. 4. Cumulative penetration curves of triamcinolone acetonide, delivered from its saturated 1 : 1 propylene glycol-water solution to untreated and enhancer treated (1 : 1 α-bisabolol/propylene glycol) human epidermis at 32°C (example plots). The left ordinate refers to the treated skin and the right ordinate refers to the untreated skin.

In this case, the stratum corneum-vehicle partitioning of the drug was not determined as both the stratum corneum and the vehicle might change throughout the incubation period, due to redistribution of BIS. In order to obtain an indication of any possible effect of BIS in shifting the drug partitioning in favour of the treated skin, the saturation solubility of TACA in the enhancer (BIS) was determined and compared with its solubility in the vehicle (1 : 1 PG/water). It was found that the enhancer/vehicle saturation solubility ratio of TACA is 3.18 (2.11 and 0.664 mg/ml, in BIS and in PG/water, respectively). This indicates that there may be some effect of BIS on the

TABLE 5

Penetration parameters of triamcinolone acetonide delivered to untreated and treated human epidermis from its saturated solution [a] in propylene glycol : water 1 : 1

Pretreatment	N [b]	Flux ($\mu g\ cm^{-2}\ h^{-1}$) ($\pm$SE)	K_p ($cm\ h^{-1}$)
None	7	0.012 ± 0.001	1.81×10^{-5}
1 : 1 PG/BIS	9	0.884 ± 0.100	1.33×10^{-3}

[a] 664 $\mu g\ ml^{-1}$.
[b] Number of runs.

TABLE 6

Effects of penetration enhancers on the transepidermal penetration of 5-fluorouracil and triamcinolone acetonide at 32°C

Penetrating drug	Enhancer	Enhancement ratio [a]
5-FU	PG	1.7
5-FU	BIS	5.4
5-FU	1:1 BIS/PG	16.9
TACA	1:1 BIS/PG	73.5

[a] Defined as: K_p (treated)/K_p (untreated).

TACA partitioning into the skin but that the potential increase in TACA solubility in the modified skin should not alone produce a 74-fold increase in penetration (see Table 6). Thus, also for this drug, increased lipid fluidity seems to play a major role in the enhancement mechanism.

It is also interesting to note that the enhancement ratios correlate negatively with the penetration rates of the various permeants through the untreated skin. For TACA, the poorest skin permeant in this study, the highest enhancement ratio was observed. A lower but still impressive enhancement ratio was calculated for 5-FU which is a better skin permeant. An enhancement ratio of only 1:6 was determined in preliminary experiments with ibuprofen which was found to penetrate relatively fast through the untreated skin ($J_{max} = 17\ \mu g\ cm^{-2}\ h^{-1}$ from 2% suspension in Carbopol gel, pH 5.5). Furthermore, none of the enhanced fluxes measured here seems unreasonably high. For example, the enhanced TACA flux noted in this study is similar to that obtained for this drug when delivered from an infinite dose of a hydroalcoholic tincture, where the enhancement effect arose from the ethanol flux (Kadir et al., 1989).

Thus, α-bisabolol holds considerable promise as a clinically acceptable penetration enhancer for poorly diffusible drugs and may be of particular value in enhancing the transdermal penetration of problem species such as peptides. A particularly valuable feature of the accelerant is its non-irritant and non-toxic effects in viable skin.

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