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Influence of penetration enhancers on the blanching intensity of betamethasone 17-benzoate

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Abstract

The influence of various potential lipophilic penetration enhancers on the blanching intensity of the model drug betamethasone 17-benzoate is examined with respect to the thermodynamic activity of the drug in the vehicle. The parameter of response is the relative intensity of blanching. The penetration enhancing effects of the vehicles are quantified using two different parameters. The first is the activity-standardized bioavailability factor determined from activity-response lines, which equals the enhancement ratio. The highest value found was 6.4. The second parameter is the activity-standardized vertical distance, that is the difference of the response of preparations with equal thermodynamic drug activity, determined from activity-response lines and from one-point measurements applying suspensions. Both penetration enhancement-describing parameters show a linear correlation with the solubility of the model drug in different vehicles. This requires a substantial penetration of the vehicles themselves to be able to act as cosolvents in the stratum corneum. Penetration enhancement by this mechanism is only possible if the drug shows differentiated affinities to lipophilic, stratum corneum-like vehicles. Betamethasone 17-benzoate formulations with low drug solubility, solutions as well as suspensions with a low amount of solid in the vehicles, show drug depletion effects.

Keywords: Penetration enhancers; Thermodynamic drug activity; Betamethasone 17-benzoate; Skin blanching; Activity-response lines

1. Introduction

The quantification of enhancing effects on drug penetration is only possible either by the direct determination of drug fluxes with respect to the thermodynamic drug activity in the vehicle or by an indirect determination through the measurement of the pharmacodynamic response (Bach and Lippold, 1998).

The dependence of the pharmacodynamic response on the applied thermodynamic drug activ-

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ity in the vehicle is described by activity-response curves. The intensity of the response is plotted versus the logarithm of the drug activity. In the absence of penetration enhancing effects congruent symmetrical curves with a sigmoidal shape will result. The linear parts are referred to as activity-response lines. In the case of penetration enhancement, the respective activity-response curve is shifted to the left, i.e. despite equal activity a stronger response is observed. Furthermore, the response plateau is elevated, meaning that even the response observed with saturated solutions and suspensions is stronger if the plateau value is limited by the drug flux (Bach and Lippold, 1998).

The quantification of penetration enhancing effects is possible by horizontal and vertical comparison of the activity-response lines. In contrast to the quantification of thermodynamic effects, the quantification of penetration enhancing effects is possible even in the plateau range of the maximum response. The activity-standardized bioavailability factors f_a, determined from the horizontal distance between the activity-response curves, correspond to the relative permeabilities at a defined drug activity and are therefore identical to the enhancement ratio defined by Goodman and Barry (Goodman and Barry, 1988; Bach and Lippold, 1998).

The influence of various potential lipophilic penetration enhancers on the blanching (Barry, 1983) of betamethasone 17-benzoate is examined with respect to the thermodynamic drug activity in the vehicle. The different methods of quantification are compared.

2. Materials and methods

2.1. Penetration enhancers or vehicles and model drug

The following lipophilic vehicles, arranged in ascending order according to their ability to dissolve the model drug betamethasone 17-benzoate (BMB; Gödecke, Berlin, Germany), were used: polyethylene oleogel (PO; 95% heavy mineral oil, 5% low density polyethylene; Bristol-Myers Squibb, Regensburg, Germany), light mineral oil (MO; Parafluid Mineralölgesellschaft, Hamburg, Germany), oleic acid (OAc; Henkel, Düsseldorf, Germany), medium chain triglycerides (MCT; Hüls, Witten, Germany), oleic alcohol (OAl; Henkel, Düsseldorf, Germany), Phospholipon® 80 (containing $>80\%$ phosphatidylcholine and up to 10% phosphatidylethanolamine; Nattermann Phospholipid, Köln, Germany) 5% in medium chain triglycerides (P80), dibutyl adipate (DBA; Henkel, Düsseldorf, Germany), (-)-α-bisabolol (Bis; BASF, Ludwigshafen, Germany).

Medium chain triglycerides were selected as standard because they were expected not to exert specific vehicle effects (Leopold and Lippold, 1995b).

The corticosteroid betamethasone 17-benzoate was used as model drug because it shows different thermodynamic activities in lipophilic solvents and penetrates slowly in spite of its relatively high lipophilicity ($PC_{oct/w} = 13\,690$; Schneemann, 1983).

2.2. Preparation of formulations with defined thermodynamic BMB activity

The BMB activities used for the generation of the activity-response lines may be taken from Table 1, the BMB solubilities (Bach, 1995), which are the basis for the calculation (Bach and Lippold, 1998) from Table 2. The required amount of BMB was dissolved in the liquid vehicles. Subsequently, the preparations were gelled with 15% highly porous polypropylene (Accurel® EP 100; Akzo Faser, Obernburg, Germany) to reduce the spreading of the vehicles.

The suspensions contain twice the saturation concentration of BMB. In the case of light mineral oil and polyethylene oleogel preparations a 0.1% concentration of BMB was also used. The

Table 1
Investigated BMB activities, expressed as percentage of solubility

$a[\%c_s]$	95.0	45.7	21.9	10.5	5.0
log a	1.98	1.66	1.34	1.02	0.70

$a[\%c_s] = c \times 100/c_s$.

Table 2
Solubilities of BMB in lipophilic vehicles at 32°C and solubility parameters δ of these vehicles calculated according to Small and Fedors (Barton, 1983)

Vehicle		c_s (mg/100 g)	δ ($MPa^{0.5}$)
PO	Polyethylene oleogel	0.15	16.2[a]
MO	Light mineral oil	0.41	16.0[b]
OAc	Oleic acid	130	17.6
MCT	Medium chain triglycerides	178	18.3
OAl	Oleic alcohol	571	18.5
P80	Phospholipon® 80	1012	—
DBA	Dibutyl adipate	1025	18.6
Bis	(-)-α-bisabolol	1087	19.6

—, not calculable, fragments are missing.
[a] Calculated as C35 iso mineral oil, MG 493.
[b] Calculated as C23 iso mineral oil, MG 325.

saturation concentrations are exceeded by factor of 2.4×10^2 and 6.7×10^2, respectively (Bach, 1995).

2.3. In vivo experiments

The blanching response of BMB is determined with the vasoconstrictor test. The test design, modified by Barry and Woodford (1978), was adopted and adjusted to the given conditions. In terms of the FDA Interim Guidance 'Topical Corticosteroids' (FDA, 1992), the 'Dilution Method' was used.

The vehicles OAc, OAl, P80, DBA and Bis were compared with the reference vehicle MCT using data from activity-response lines and by the determination of the response with suspensions. Only the activity-response lines of two vehicles, test and standard, may be obtained with each volunteer. If suspensions were applied, all vehicles may be tested with each volunteer which allows paired observations.

Furthermore, activity-response lines of MO and DBA were compared and BMB suspensions with increasing amounts of solid in MO and PO were applied with MCT as reference. Blank preparations were always applied to ensure that there was no placebo effect.

Activity-response lines were obtained from 12 (or six) human volunteers, whereas for the application of suspensions 24 human volunteers were examined. At most, 12 preparations (including the BMB-free blanks) were applied to each volunteer in quadruplicate, which leads to 48 application sites. PETP (polyethylene terephthalate) membranes, laminated with Duplomed® 2806 double-sided adhesive tape (Lohmann, Neuwied, Germany), 5.5×13.5 cm^2, were used to mark the location of the application sites. From these membranes 7×7 mm squares leading to application areas of 0.49 cm^2 were cut out in a symmetrical arrangement. One membrane was fixed to the centre of the ventral side of each forearm of the subjects; 10 ± 1 mg of the preparations were weighed on glass spatulas and randomly assigned to the application sites in a double-blind manner. The applied preparations were covered occlusively with laminated PETP membranes (see above). To hold them in place, Fixomull stretch® (Beiersdorf, Hamburg, Germany) was used (Bach, 1995); 12 h after the application of the preparations, the membranes and the residues of the preparations on the skin were removed. The skin was cleaned with water and Nivea® shower gel 'Vital-Pflege'; 14, 16, 18, 20 and 22 h after application the blanching was visually quantified by one observer according to a scoring scale (0, no; 1, slight; 2, medium, 3, strong blanching). Half points were also used to differentiate the blanching. Daylight served as the light source. This method was not combined with the application of a chromameter as proposed in the FDA Interim Guidance (FDA, 1992).

The scoring scale by Barry and Woodford was unsuitable because the spreading of the lipophilic vehicles cannot be completely prevented. Therefore, blanching outside the treated areas may be observed. The application of a smaller amount of preparation was unsuitable since on the one hand the spreading of the lipophilic vehicles cannot be completely prevented and on the other hand the risk of depletion effects increases. The scoring scale was adopted from Meyer et al. (1981).

Volunteers that did not reach 50% of the maximum blanching (1.5 points) with any preparation are not included in the data analysis. For the

purposes of this study, 150 human volunteers (96 females, 54 males) in the age range of 17–40 years were examined; 30 volunteers (20%) could not be included in the data analysis. They either showed too low a blanching response and therefore did not reach the required score (27 volunteers) or they reacted allergically to the adhesive tape (2 volunteers). In one case the membranes peeled off during the time period of application. None of the volunteers showed eczematous or allergical skin reactions or underwent medical treatment of the skin. The examinations were approved by the ethical committee of the medical faculty of the University Hospital in Düsseldorf. Medical supervisor was Professor Dr G. Goerz, Dermatology Department of the University Hospital. All volunteers gave their written consent after having been informed about the details of the study design.

2.4. Data treatment

The blanching response may be quantified according to Eq. (1): A_T and A_{St} are the mean scores of the fourfold application of the test and the standard preparation (MCT or DBA, $c_{BMB} = 95\% c_s$ or $2c_s$) at the response maximum, respectively.

$$\text{Relative blanching intensity} \quad A_{rel}[\%] = \frac{A_T}{A_{St}} \cdot 100 \tag{1}$$

A normal distribution of the data is assumed here. To examine the homogeneity of variance of the response data, the Bartlett test is performed with the data of the activity-response lines for the vehicles OAc, OAl, P80, DBA, Bis and MCT as reference vehicle. The data A_{rel} show homogeneity of variance in 60% of the data (OAl, P80, DBA). The scoring scale is not used in its full range as a result of the only moderate blanching in the majority of the volunteers. Thus, a response of 100% does not necessarily correspond to the upper limit of the scoring scale.

For the generation of the activity-response lines only the relative blanching intensities A_{rel} at maximum response are used. Blanching intensities not differing from zero are not used for the calculation of the regression lines, provided that blanching has not already been observed at lower activities. The logarithm of the activity-standardized bioavailability factor f_a is calculated according to Eq. (2) (Lippold and Schneemann, 1984). Mathematically, it is the horizontal distance of two first-order regression lines, i.e. the underlying population is normally distributed. Preconditions are statistically equal deviations and regression coefficients as well as unequal y-axis intercepts (Wissenschaftliche Tabellen Geigy, 1980). The activity-standardized bioavailability factor f_a corresponds to the enhancement ratio ER according to Goodman and Barry (1988).

$$\log f = \bar{x}_R - \bar{x}_T - \frac{\bar{y}_R - \bar{y}_T}{\bar{b}_{xy}} \tag{2}$$

where $\bar{x}_R$ and $\bar{x}_T$ are the mean values of the logarithms of the activities of the reference or test preparation; $\bar{y}_R$ and $\bar{y}_T$ are the mean responses of the reference or test preparation, and $\bar{b}_{xy}$ is the mean regression coefficient of the two regression lines (Wissenschaftliche Tabellen Geigy, 1980).

Statistical inequality of the regression coefficients and the y-axis intercepts is rarely found because of the scattering of the in vivo data. Hence, it is assumed that the requirements are fulfilled.

The logarithms of the activity-standardized bioavailability factors $\log f_a$ are calculated for each volunteer. Subsequently, the statistical parameters ($\bar{x}$, 95%-confidence intervals) are computed and the inverse logarithms are calculated from the resulting values. The activity-standardized vertical distance d_a (response difference at equal thermodynamic activities) is calculated according to Eq. (3) in the case of the activity-response lines (Wissenschaftliche Tabellen Geigy, 1980) and according to Eq. (4) in the case of the application of suspensions. As with the logarithms of the activity-standardized bioavailability factors, the activity-standardized vertical distances are calculated for each volunteer.

$$d_a = \bar{y}_R - \bar{y}_T - \bar{b}_{yx} \cdot (\bar{x}_R - \bar{x}_T) \tag{3}$$

$$d_a[\%] = A_{rel} - 100\% \tag{4}$$

where $\bar{b}_{yx}$ is the mean regression coefficient of the two regression lines (Wissenschaftliche Tabellen Geigy, 1980).

3. Results and discussion

3.1. Activity-standardized bioavailability factors, cosolvent effect

The time course of the blanching response is exemplarily shown in Fig. 1. The blanching intensity increases continuously after removing the membranes and reaches a maximum 18 h after application of the preparations. The time until the response maximum is reached, t_{max}, is a function of the penetration rate constant, i.e. in the case of penetration enhancement a decrease of t_{max} and in the case of penetration retardation an increase of t_{max} is expected. Contradicting results have been obtained for betamethasone 17-benzoate. In contrast to Hackemüller (1988), Schneemann observes no influence of the penetration rate constant on t_{max} (Lippold and Schneemann, 1984).

Fig. 2 shows the activity-response lines for the relative blanching intensities at the maximum response (18 h after application of the preparations) of BMB application in DBA and MCT. For the tested vehicles with MCT as reference activity-standardized bioavailability factors f_a of up to 6.4 (P80) are obtained (Table 3). This means a 6.4-fold penetration enhancement for BMB is found at maximum, i.e. the product $D_B.c_{sB}$ is increased as a result of penetration enhancement by a factor of 6.4.

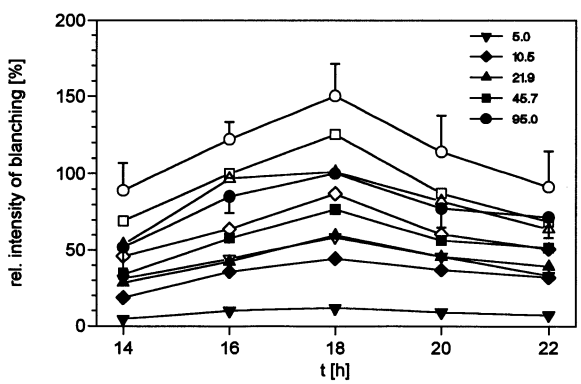

Fig. 1. Blanching intensity–time relationship for the application of BMB in DBA (open symbols) and MCT (solid symbols) depending on the BMB activity [%c_s], $\bar{x}$ resp. $\bar{x}+95\%$, confidence intervals (○) resp. $\bar{x}\pm95\%$ confidence intervals (●), $n=12$.

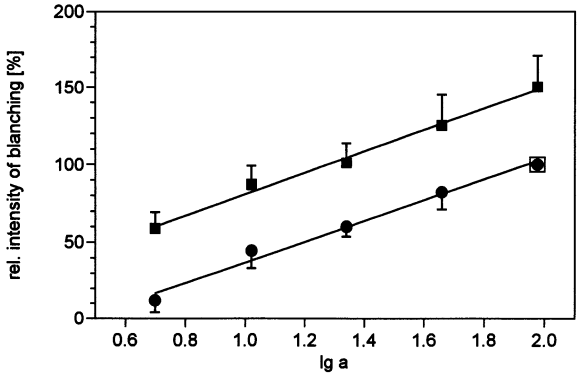

Fig. 2. Activity-response lines for the application of BMB in DBA (■) and MCT (●, □ standard) for $t=18$ h, a as [%c_s], $\bar{x}+95\%$ confidence intervals (■) resp. $\bar{x}-95\%$ confidence intervals (●), regression lines, $n=12$.

In the case of the 12-h treatment with oleic acid, it has to be considered that 10 out of 12 volunteers show reddish or brownish skin reactions that partly cover the application area. Therefore, estimation of the blanching response is possible, but is more difficult or even falsified. If the skin is cleaned with isopropyl alcohol after the test, the brownish spots disappear or clearly become weaker. In some cases, the skin irritations remain for several days; 18-h treatment but not 12-h treatment with oleic acid in propylene glycol leads to skin exfoliation in rabbits (Hsu et al., 1991). The general requirement that a penetration enhancer has to be non-irritant is not fulfilled in the case of oleic acid. In general, the results of the experiments with oleic acid are listed, but are not included in the correlations.

Table 3
Activity-standardized bioavailability factors f_a from activity-response lines, reference MCT ($f_a=1$)

Vehicle	f_a	
	$\bar{x}$	95% confidence interval
OAc	0.22	0.14–0.28
OAl	2.5	1.9–3.2
P80	6.4	5.2–8.3
DBA	4.7	3.7–6.3
Bis	4.0	2.9–5.3

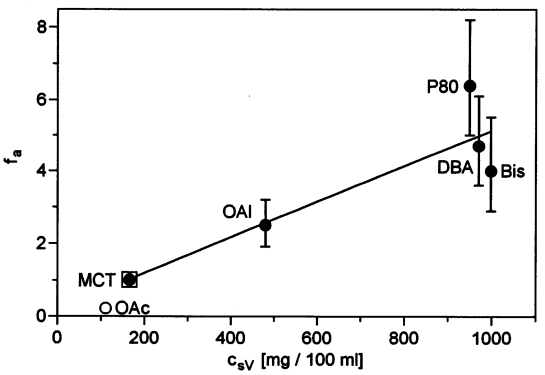

Fig. 3. Activity-standardized bioavailability factors f_a for BMB in dependence on the drug solubility in the potential enhancers c_{sV}, $\bar{x} \pm 95\%$ confidence intervals, regression line, $n = 12$, □ reference.

There is evidence that activity-standardized bioavailability factors f_a above 4 are only obtained with vehicles which show relatively high betamethasone 17-benzoate solubilities up to 1%. Overall, the penetration enhancing effect seems to correlate with the betamethasone 17-benzoate solubility in the enhancers. Fig. 3 shows the relationship between the activity standardized bioavailability factors (y-axis) and the solubility in the enhancers (x-axis). However, it has to be proven whether the relative deviations from the regression line are equal from a statistical point of view, that means whether linearity is given. For this reason, the activity-standardized bioavailability factors f_a are divided by the relative betamethasone 17-benzoate solubilities. This relative solubility is obtained by division of the solubility in the potential enhancer by the solubility in medium chain triglycerides. If these ratios of f_a and the relative solubility are plotted versus the solubility in the enhancers (Fig. 4), a straight line with a slope of zero should result, that means the quotients should not differ statistically. Indeed, no significant difference can be found if the ratio of f_a and the relative solubility for MCT are compared with the respective ratios for the other investigated potential penetration enhancers using the one sample t-test. Exceptions are oleic acid ($p = 0.001$) and α-bisabolol ($p = 0.05$). Thus, the penetration enhancing effect of the potential enhancers increases in a linear manner with the solubility of the model drug in the vehicle. The blanching response of BMB with oleic acid as vehicle is too low in relation to its solubility. This observation is most likely due to the above-mentioned skin irritation.

A probable mechanism of the penetration enhancement is the substantial penetration of the enhancers into the barrier stratum corneum and the increase of the solubility c_{sB} of betamethasone 17-benzoate there leading to a higher permeability P_B. Thus, they act as cosolvents in the stratum corneum. Consequently, the extent of penetration enhancement is not only dependent on the solubility of the drug in the enhancer, but also on the extent of enhancer penetration. Even with various non-steroidal anti-inflammatory drugs in different vehicles, Wild (1988) discovered that the penetration tendency of the drugs depends on their solubility in the vehicle and on the vehicle penetration capacity, i.e. their spreading on the skin.

As with oleic acid, the blanching response of BMB with α-bisabolol as vehicle is too low in relation to its solubility. This could be due to a lower penetration tendency of the vehicle α-bisabolol in comparison to the other vehicles. However, it has to be considered that α-bisabolol acts as an anti-inflammatory agent and is therefore not pharmacologically inert. Meanwhile, there seems to be evidence that the anti-inflammatory response of non-steroidal anti-inflammatory drugs

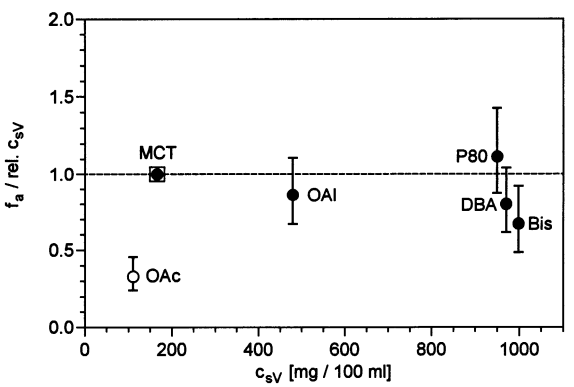

Fig. 4. Ratio of the activity-standardized bioavailability factors f_a for BMB and the relative drug solubility c_{sV} in dependence on the drug solubility in the potential penetration enhancers c_{sV}, $\bar{x} \pm 95\%$ confidence intervals, $n = 12$, □ reference.

is not only due to the inhibition of prostaglandin synthesis. The signal transduction at cell membrane level may be inhibited by accumulation of the drugs in the lipophilic membranes and binding to defined proteins, i.e. G-proteins (Abramson and Weissmann, 1989). According to Haigh and Kanfer (1984), a probable mechanism for skin blanching induced by corticosteroids is the binding to a G-protein associated receptor with subsequent liberation of intracellular mediators such as adenosine-3′,5′-monophosphate or guanosine-3′,5′-monophosphate. Therefore, it cannot be ruled out that the lower betamethasone 17-benzoate response is a result of the inhibition of the synthesis of these mediator substances by α-bisabolol.

In the literature the following mechanisms of penetration enhancement are discussed: increase of the drug partition coefficient $PC_{B/V}$ (Watkinson et al., 1990; Okamoto et al., 1991; Shah et al., 1992; Kim et al., 1993; Lee et al., 1993; Koyama et al., 1994) or of the solubility in a model lipid (Sasaki et al., 1991) and increase of the diffusion coefficient in the barrier stratum corneum D_B (Williams and Barry, 1991; Shah et al., 1992; Lee et al., 1993; Kobayashi et al., 1994; Koyama et al., 1994; Leopold and Lippold, 1995a). Increased partition coefficients are found when applying drug suspensions (Shah et al., 1992; Kim et al., 1993; Lee et al., 1993) or pretreating the skin with enhancer (Watkinson et al., 1990; Okamoto et al., 1991; Koyama et al., 1994), i.e. under the conditions of equal drug activities. Therefore, the increased $PC_{B/V}$-values are a result of an increased drug solubility in the modified barrier c_{sB}.

Lipid fluidization is often mentioned as a mechanism of penetration enhancement. The consequence may not only be the increase of the diffusion coefficient D_B, but also of the solubility in the barrier c_{sB} (Bach and Lippold, 1998). Recently, even the formation of separate phases in the stratum corneum has been postulated for oleic acid and some terpenes (Francoeur et al., 1990; Ongpipattanakul et al., 1991; Walker and Hadgraft, 1991; Cornwell et al., 1994). The increased solubility in these separate phases could lead to an overall increase of the drug solubility in the stratum corneum. Altogether, the concept that only small polar penetration enhancers can act as cosolvents in the stratum corneum (Barry, 1991) has to be reconsidered. Even lipophilic penetration enhancers with solubility parameters close to that of the stratum corneum ($\delta \sim 20.5$ MPa$^{0.5}$; Liron and Cohen, 1984; Sloan et al., 1986) may act as cosolvents in the stratum corneum.

The solubility parameter of betamethasone 17-benzoate ($\delta = 19.0$ MPa$^{0.5}$; Schneemann, 1983) is also close to that of the stratum corneum. The BMB solubility reaches its maximum in those potential penetration enhancers with the most similar solubility parameters (Table 2). Therefore, the greatest enhancing effects are achieved with those substances showing solubility parameters that are close to that of BMB and thus to that of the stratum corneum. Vehicles with solubility parameters between 16.4 and 24.6 MPa$^{0.5}$ have been shown to increase the skin permeability for theophylline ($\delta = 28.6$ MPa$^{0.5}$) but not for salicylic acid ($\delta = 22.1$ MPa$^{0.5}$) due to lipid extraction (Sloan et al., 1986). The potential penetration enhancers examined in this study ($\delta = 16.9$–21.7 MPa$^{0.5}$) should rather increase the penetration of ethyl nicotinate ($\delta = 22.7$; Le, 1993) than that of betamethasone 17-benzoate. However, the opposite is the case. The penetration of ethyl nicotinate, quantified as the reciprocal value of the relative latency time until the onset of the erythema, cannot be enhanced by lipophilic potential penetration enhancers (Bach, 1995). Nevertheless, with direct flux measurements, a slight penetration enhancement could be found for methyl nicotinate (Leopold and Lippold, 1995b). Principally, not only the solubility parameter of the drugs but also their physico-chemical properties have to be considered.

Ethyl nicotinate shows higher but less differentiated affinities to the potential enhancers and mineral oil-containing vehicles (the relative thermodynamic activity coefficients $\gamma_{T/St}$ (Bach and Lippold, 1998) only differ by a factor of 12.5 (Bach, 1995)) than betamethasone 17-benzoate ($\gamma_{T/St}$-values differ by a factor of 7.7×10^3). Although, the solubility parameter of ethyl nicotinate deviates more from those of the vehicles than that of betamethasone 17-benzoate.

Only for drugs that show differentiated solubilities in different lipophilic media, potential penetration enhancers may either serve as cosolvents in the stratum corneum (high drug solubility) or lead to lipid fluidization in the stratum corneum which results in increased drug solubility (low drug solubility). For drugs such as ethyl nicotinate that do not show this differentiated solubility in potential lipophilic penetration enhancers and stratum corneum lipids, the solubility in the stratum corneum can only be influenced to a limited extent. Therefore, a penetration enhancing effect for ethyl nicotinate is only found if the skin is pretreated with occluding mineral oil-containing vehicles. These vehicles probably lead to an increased diffusion coefficient of ethyl nicotinate in the barrier stratum corneum D_B due to hydration of the stratum corneum (Bach, 1995).

Since the blanching tests were carried out under occlusive conditions, there is no statement possible concerning the influence of occluding vehicles such as polyethylene oleogel on the penetration of betamethasone 17-benzoate. However, penetration enhancement due to occlusion has to be expected for betamethasone 17-benzoate since non-occlusively applied preparations lead to a less pronounced blanching response (Barry and Woodford, 1978).

3.2. Activity-standardized vertical distances

Activity-response lines also enable the determination of the activity-standardized vertical distances d_a. The activity-standardized vertical distances may be correlated with the activity-standardized bioavailability factors f_a (Fig. 5). Here, oleic acid may be included in this correlation because the inaccuracy in the determination of the blanching response occurs with both response parameters.

The correlation is very good. However, for the response parameters different ranking orders of enhancement result depending on which factor is taken as a basis (f_a or d_a). This is due to the fact that the mean common regression coefficients of the two regression lines of the test and reference preparations differ from each other if the single individual is looked at. In the case of identical

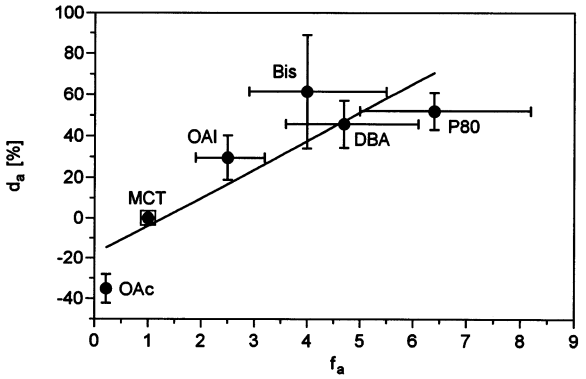

Fig. 5. Correlation of the activity-standardized vertical distances d_a with the activity-standardized bioavailability factors f_a, both determined from activity-response lines, $\bar{x} \pm 95\%$ confidence intervals, $\bar{y} \pm 95\%$ confidence intervals, regression line ($y = -17.8 + 13.8x$), $r = 0.88$, □ reference.

activity-standardized bioavailability factors f_a the activity-standardized vertical distance d_a becomes greater, the more the regression coefficient increases.

As can be seen in Fig. 5, an activity-standardized bioavailability factor f_a of about 5 corresponds to an activity-standardized vertical distance d_a of about 50%. An activity-standardized bioavailability factor f_a within this range is found for dibutyl adipate with medium chain triglycerides as reference. The respective activity-response lines are shown in Fig. 2. With regard to the standard, i.e. the reference preparation with a relative thermodynamic drug activity of 0.95 ($c = 95\%c_s$), the test preparation with the same thermodynamic activity shows a response improvement by a factor of ~ 1.5 (test 150%, standard 100% response). For every thermodynamic activity level (e.g. $c = 5\%c_s$) the same activity-standardized vertical distance results (50%) using the same standard as reference. However, if for the calculation of the activity-standardized vertical distances the reference preparations with equal thermodynamic activities are used, activity-standardized vertical distances greater than 50% result. The smaller the thermodynamic drug activity in the vehicle becomes, the greater is the resulting response improvement factor. In the example given, a response improvement factor of about 4 is found for a relative thermodynamic

activity of 0.05 ($c = 5\%c_s$). Therefore, the choice of the standard vehicle is of importance in the case of one-point measurements if the influence of potential penetration enhancers on the response of a model drug is examined at different drug activities and if the response improvement factors are compared.

The activity-standardized vertical distances d_a are of greater clinical relevance than the activity-standardized bioavailability factors f_a but they give no information on the influence of the penetration enhancers on the permeability of the barrier P_B and the product $D_B.c_{sB}$, respectively.

In contrast to the determination of the activity-standardized bioavailability factor f_a, the determination of the activity-standardized vertical distance d_a is also possible with one-point measurements, i.e. the determination of the pharmacodynamic response at a given thermodynamic drug activity, even at maximum activity. However, a precondition is that the response is only flux-limited (Bach and Lippold, 1998). Therefore, the potential penetration enhancers, already characterized by activity-response lines, are applied as BMB suspensions, i.e. with maximum BMB activity. The BMB suspension in MCT serves as standard. The relative blanching intensities at the maximum blanching response (18 h after application of the preparations) are used for the calculation of the activity-standardized vertical distances. These activity-standardized vertical distances from one-point measurements should correlate with those from activity-response lines. Fig. 6 shows the respective plot. The correlation coefficient r is 0.94 which means that a good correlation is given. The regression coefficient b of the regression line is only 0.83, i.e. the suspensions in general show slightly smaller activity-standardized vertical distances. A regression coefficient of $b = 1$ would have been expected. For the activity-response lines and suspensions different standards are used with regard to their drug activity. While the thermodynamic drug activity of the standard is 95% ($\log a = 1.98$) in the case of the activity-response lines, it is maximum for the suspensions (100%, $\log a = 2.00$). Since the logarithms of the activities differ only marginally and the activity-response lines are flat, no statistically different response should result. Therefore, the difference between the drug activities of the standards should not be the reason for the lower activity-standardized vertical distances observed with the suspensions.

The quantification of blanching with the help of a scoring scale is more difficult in the case of one-point measurements than it is in the case of activity-response lines because a less differentiated blanching response has to be quantified. For activity-response lines, differentiated blanching is obtained when using different activities, even if vehicles with only weak penetration enhancing properties are examined.

However, the activity-standardized vertical distances from activity-response lines and from one-point measurements are in the same range and generally lead to the same conclusion.

3.3. Drug depletion effects

Fig. 7 shows the activity-response lines for the application of BMB in light mineral oil and dibutyl adipate, respectively. The BMB solubility in light mineral oil is very low (0.41 mg/100 g).

While the BMB preparations in DBA show a linear dependence of the response parameter on the logarithm of the drug activity, the preparations in light mineral oil virtually show no re-

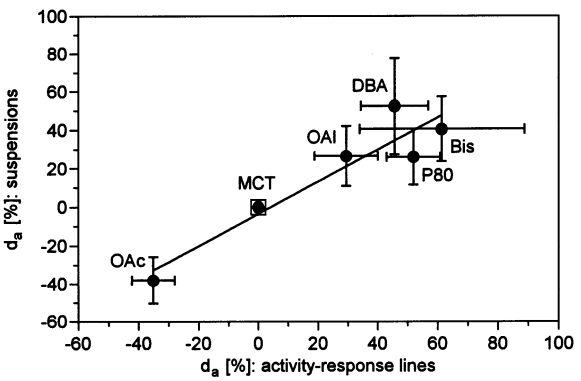

Fig. 6. Correlation of the activity-standardized vertical distances d_a determined from one-point measurements by application of suspensions with those from activity-response lines, $\bar{x} \pm 95\%$ confidence intervals, $\bar{y} \pm 95\%$ confidence intervals, regression line ($y = -3.40 + 0.83x$), $r = 0.94$, □ reference MCT.

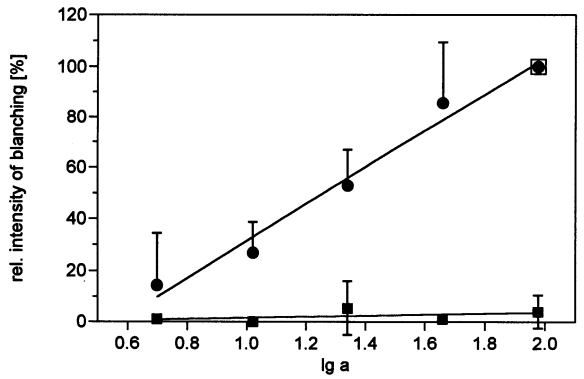

Fig. 7. Activity-response lines for the application of BMB in MO (■) and DBA (●, □ standard) for $t = 18$ h, a as [%c_s], $\bar{x} + 95\%$ confidence intervals (■) resp. $\bar{x} - 95\%$ VB confidence intervals (●), regression lines, $n = 6$.

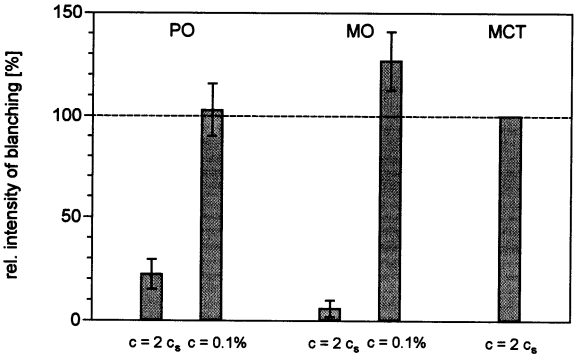

Fig. 8. Relative blanching intensities after application of BMB suspensions in mineral oil-containing vehicles and the reference vehicle MCT for $t = 18$ h, $\bar{x} + 95\%$ confidence intervals, $n = 24$.

sponse. The reason may be a depletion of the preparations as has been found for methyl nicotinate and even for betamethasone 17-benzoate (Lippold and Schneemann, 1984; Lippold and Reimann, 1989; Malzfeldt et al., 1989). In the case of high drug solubilities in the vehicle, the drug penetration rate is relatively low at defined concentrations (e.g. 50% c_s or c_s) or relative thermodynamic drug activities (0.5 or 1.0). Therefore, the concentration in the vehicle decreases only slightly and the relative thermodynamic drug activity in the vehicle almost remains constant. In the case of low drug solubilities the same penetration rate may lead to a drastic decrease of the drug concentration in the vehicle. The relative thermodynamic drug activity in these preparations decreases faster than in preparations with high drug solubilities.

To confirm this hypothesis examinations with BMB suspensions in light mineral oil and polyethylene oleogel with different amounts of solid are carried out. In suspensions the drug reaches its maximum thermodynamic activity and therefore the maximum response may be observed. If in the case of drug suspensions the flux or the response increases with the amount of solid, drug depletion of suspensions with a low amount of solid will occur.

Fig. 8 shows the relative blanching intensities at maximum response obtained with the investigated vehicles. It is obvious that the suspensions with only the twofold saturation concentration of BMB in light mineral oil and polyethylene oleogel show drug depletion. The relative blanching intensities are 22% for polyethylene oleogel and below 10% of the standard value for light mineral oil, respectively. In contrast, in the case of the 0.1% preparations of BMB the response is comparable to that of the standard preparation and even better, respectively. The dissolution rate that may become the rate limiting step (Barrett et al., 1965; Lippold, 1992), does not seem to be of importance. This effect however cannot be ruled out for BMB batches with a larger particle size.

A penetration enhancing effect may be postulated for light mineral oil as compared to the standard MCT. So far penetration enhancing effects for light mineral oil have rarely been observed. Leopold observed penetration enhancement of light mineral oil for methyl nicotinate (Leopold and Lippold, 1995b). DSC measurements show that this penetration enhancement is attributed to lipid fluidization and probably extraction or dissolution of the stratum corneum lipids (Leopold and Lippold, 1995a) which may lead to an increase of the diffusion coefficient in the barrier D_B.

4. Conclusions

Penetration enhancing effects for BMB, examined by determination of the blanching response,

may be quantified by the generation of activity-response lines as well as by one-point measurements. As a result of the flatness of the activity-response lines the penetration enhancing factors from horizontal comparison (activity-standardized bioavailability factors f_a) are greater than those obtained by vertical comparison (activity-standardized vertical distances d_a). Therefore, penetration enhancing effects may be identified more accurately by horizontal comparison of the activity-response lines. In addition, the activity-standardized bioavailability factors f_a allow us to draw conclusions with regard to the changes of the product $D_B.c_{sB}$. Solution-type preparations and suspensions with a low amount of solid with light mineral oil as vehicle show drug depletion effects. The BMB solubility in light mineral oil is below 1 mg/100 ml. The generation of activity-response lines with light mineral oil as vehicle is therefore useless. Suspensions in polyethylene oleogel with a low amount of solid also show drug depletion effects.

The penetration enhancing effects of the examined vehicles on BMB penetration are probably due to the penetration of the enhancers into the barrier stratum corneum to a high extent and the resulting increase of the solubility of BMB c_{sB} there. The only exception is light mineral oil.

Penetration enhancing effects caused by an increase of c_{sB} are either possible through cosolvents, as shown here for betamethasone 17-benzoate, or by structural changes of the stratum corneum such as lipid fluidization as a result of the penetration of even small amounts of enhancer. A basic requirement for increasing c_{sB} is that the drug shows differentiated solubility properties or rather affinities to lipophilic stratum corneum-like vehicles.

The diffusion coefficient in the stratum corneum D_B and its alteration seems to be less dependent on the properties of the drug (Bach and Lippold, 1998).

In particular, pronounced penetration enhancing effects should rather result from the increase of the drug solubility in the barrier stratum corneum c_{sB} than from the increase of the drug diffusion coefficient in the barrier stratum corneum D_B (Bach and Lippold, 1998). Since substances with similar solubility parameters may show very different solubility properties, a lipophilic, hydrophilic or universal model drug for enhancement predictions does not exist. The usefulness of potential penetration enhancers for a well-defined drug has always to be proven in each individual case.

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