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The Maximum Pharmacodynamic Effect as a Response Parameter: Pharmacokinetic Considerations

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Abstract

In previous human in-vivo studies measuring the maximum pharmacodynamic response to characterize cutaneously applied ointment preparations, it was observed that differences between various formulations caused by penetration enhancement led to different enhancement factors depending on the method used for determination of these factors from activity–reponse curves. To clarify this discrepancy, pharmacokinetic simulations have been performed based on an open one-compartment model with either first- or zero-order drug penetration kinetics and first-order elimination kinetics.

Under the assumption that the maximum pharmacodynamic response corresponds to the maximum effective drug concentration in the receptor compartment, which represents the difference between the maximum drug concentration and the threshold concentration, drug concentration vs time profiles and dose–reponse curves were simulated. In addition, maximum effective concentrations were calculated and plotted against the logarithm of the thermodynamic drug activity to obtain activity–reponse curves. Relative bioavailability and enhancement factors were determined either from the horizontal distance between the curves of a standard and a test preparation, or as the ratio of the maximum effective concentration of test and standard formulations.

A significant difference between the first-order and the zero-order input kinetics with regard to the evaluation of bioavailability and drug penetration enhancement was shown. Under finite dose conditions, i.e. first-order input kinetics from solution-type preparations, a misestimation of the factors usually occurs. Only under infinite dose conditions, i.e. if large preparation volumes are applied to achieve zero-order input kinetics, is the determination of bioavailability and enhancement factors from dose– and activity–reponse curves accurate.

Thermodynamic and penetration-enhancing effects of vehicles on the percutaneous penetration of drugs through intact human skin in-vivo may be investigated pharmacodynamically with model drugs that induce a quantifiable local response (Haleblian 1976). Pharmacodynamic effects that may be quantified are the vasodilation-induced erythema and skin temperature increase caused by nicotinic acid esters (Lippold & Teubner 1981b; Lippold & Reimann 1989b), the vasoconstriction-induced skin blanching of topical corticosteroid administration (Stoughton 1969; Barry & Woodford 1978; Lippold & Schneemann 1984; FDA 1995; Bach & Lippold 1998a), and the local anaesthesia induced by local anaesthetic bases (Leopold & Maibach 1999). The erythema observed after nicotinate application and the vasoconstriction in the case of corticosteroids have been used as quantification criteria. Various time- and intensity-related response parameters may be used to evaluate vehicles in terms of their penetration-modifying properties. The lag time of onset of an effect, used as the reciprocal value, is a suitable parameter if one model drug is looked at. As it may be affected by drug depletion from the vehicle, it should be measured under infinite dose conditions, i.e. zero-order penetration kinetics (Lippold & Reimann 1989b; Leopold 1998a, b). However, if

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different drugs of the same type are compared, such as a homologous series, this parameter has been shown to be useless (Le 1993). In the past, the duration of a response was measured to compare different drugs pharmacodynamically. Under certain circumstances, a linear relationship between the duration and the drug dose can be found (Levy 1966). However, if the duration of the effect is used to characterize different ointment preparations in terms of their penetration-modifying properties, it has to be considered that this parameter is not useful for investigating suspension-type preparations. Moreover, pharmacokinetic analysis of this parameter has shown that the phenomenon of drug depletion from the vehicle significantly affects the shape of the resulting dose–reponse curves and thus the estimation of the relative bioavailability, which makes this parameter unsuitable (Leopold 1998a).

The aim of this theoretical analysis was to evaluate pharmacokinetically a further pharmacodynamic parameter, the maximum response, as an intensity-related response parameter. From simulated dose– and activity–reponse curves bioavailability data and penetration enhancement factors were calculated and compared with results from previous in-vivo studies. In those studies the maximum response was determined either as the maximum vasodilation or skin temperature increase after application of nicotinates (Lippold & Teubner 1981b) or as the maximum skin blanching induced by corticosteroids (Lippold & Schneemann 1984; FDA 1995; Bach & Lippold 1998a).

Materials and Methods

Pharmacokinetic simulations

The following calculations were made on the basis of an open one-compartment model under the assumption that the time course of the drug concentration at the receptor site exceeding the minimum threshold concentration corresponds to that of the intensity of the pharmacodynamic response. As drug diffusion through the skin can most often be adequately described by Fick's first law of diffusion, it was assumed that drug penetration follows either first-order or zero-order kinetics. Moreover, it is believed that within the applied dose range the drug concentration in the receptor compartment does not reach saturation level with regard to the occupation of the receptors.

For first-order input kinetics the drug concentration vs time profile at the receptor site is described by the Bateman equation:

$$c = \frac{D_0}{Vd} \cdot \frac{k_{p1}}{k_{p1} - k_e} \cdot \left(e^{-k_e(t-t_{lag})} - e^{-k_{p_1}(t-t_{lag})}\right) \quad (1)$$

where c is the drug concentration at the receptor site, D_0 is the drug dose, Vd is the distribution volume at the receptor site, k_{p1} and k_e are first-order penetration and first-order elimination rate constants, respectively, t is time, and t_{lag} is the lag time of drug penetration.

From this equation the maximum drug concentration, c_{max}, at the receptor site is calculated as follows:

$$c_{max} = \frac{D_0}{Vd} \cdot \left(\frac{k_{p1}}{k_e}\right)^{\frac{k_e}{k_e - k_{p_1}}} \quad (2)$$

c_{max} is reached at the time point t_{max}, which is defined as follows:

$$t_{max} = \frac{1}{k_{p1} - k_e} \cdot \ln\left(\frac{k_{p1}}{k_e}\right) + t_{lag} \quad (3)$$

For zero-order input kinetics, drug concentration vs time profiles at the receptor site may be described as follows:

$$c = \frac{k_{p0}}{Vd \cdot k_e} \cdot \left(1 - e^{-k_e(t-t_{lag})}\right) \quad (4)$$

where the zero-order penetration rate constant k_{p0} represents the product of k_{p1} and D_0.

The maximum drug concentration c_{max} is then easily determined:

$$c_{max} = \frac{k_{p0}}{Vd \cdot k_e} \quad (5)$$

The time point t_{max} at which c_{max} is reached is dependent on the elimination rate constant and t_{lag}, and is estimated to approach five-times the elimination half-life plus t_{lag}.

For all simulations the distribution volume, Vd, and the elimination rate constant, k_e, were kept constant (10 mL and $0{\cdot}2\,h^{-1}$, respectively). However, it should be remembered that in the case of drugs which induce vasodilation or vasoconstriction, k_e might be affected by a change in the blood flow. Therefore, it is essential that only one model drug is used in a study so that the variations of the elimination constant are kept to a minimum. The first-order penetration rate constant k_{p1} was adjusted to 0·03, 0·1, 0·3, 0·5, 0·8, and $1{\cdot}2\,h^{-1}$, respectively. It was decided that the threshold concentration at the receptor site required for an effect to become obvious, $c_{min_{eff}}$, should be $0{\cdot}4\,\mu g\,mL^{-1}$. The lag times of drug penetration, t_{lag}, were assumed to be constant for a given drug.

For this study, the stratum corneum was regarded as the main barrier of penetration. For most of the

model drugs used in pharmacodynamic studies the diffusion through the stratum corneum is the rate-limiting step of drug penetration into the skin where the intercellular or transcellular routes of diffusion play the main role. Using Fick's first law of diffusion, the drug input from solution-type ointment preparations containing a freely available drug through the stratum corneum, which is regarded as a homogeneous lipophilic partition membrane, may be described as a first-order process where the penetration rate constant k_{p1} is defined as follows:

$$k_{p1} = D_B \cdot A \cdot PC_{B/V}/(d_B \cdot V_V) \quad (6)$$

where D_B is the diffusion coefficient of the drug in the stratum corneum, A is the application area, $PC_{B/V}$ is the stratum corneum/vehicle partition coefficient of the drug, d_B is the thickness of the stratum corneum, and V_V is the volume of the applied preparation.

In the case of very low penetration rate constants the drug amount in the vehicle, D_0, may be regarded as constant for a certain time period and can be multiplied by k_{p1} to obtain the zero-order penetration rate constant k_{p0}. Zero-order penetration kinetics were also observed after application of suspension-type preparations with a sufficient amount of undissolved drug, provided that drug release from the ointment was fast compared with drug penetration through the skin.

Bioavailability factor (F) and enhancement factor (EF)

If the maximum effect R_{max} (assumed to equal the maximum effective drug concentration at the receptor site $c_{max} - c_{min_{eff}}$ for a given preparation) was used as the response parameter to simulate dose–reponse curves, sigmoid curves were obtained (Lippold & Teubner 1981a; Lippold & Schneemann 1984). The plateau at high dose levels was due to the formation of a saturated drug solution (suspension), which led to the maximum drug flux, J_{max}. From these curves the relative bioavailability of a test preparation (T) compared with a standard formulation (ST) could be determined. The relative bioavailability factor (F) was defined as:

$$F = k_{p1}T/k_{p1}ST \quad (7)$$

where $k_{p1_{ST}}$ was set at $0{\cdot}03\,h^{-1}$ and it was decided that the drug solubility in the standard vehicle was to be $10\,mg\,mL^{-1}$. Bioavailability factors that are calculated according to equation 7 have been called true F values throughout this paper.

With R_{max} (the relevant $c_{max} - c_{min_{eff}}$) as a response parameter, f may be calculated as follows:

$$f = \frac{\left(c_{max_T} - c_{min_{eff}}\right) \cdot Vd \cdot k_e}{\left(c_{max_{ST}} - c_{min_{eff}}\right) \cdot Vd \cdot k_e} = \frac{c_{max_T} - c_{min_{eff}}}{c_{max_{ST}} - c_{min_{eff}}} = \frac{R_{max_T}}{R_{max_{ST}}} \quad (8)$$

This equation is mathematically correct only if the maximum effects are determined at equal dose levels below drug saturation in the vehicle, and it is applicable only in the case of zero-order penetration kinetics. Above the drug solubility limit in the vehicle any difference in R_{max} between test and standard preparations indicates a barrier-modifying action of either test or standard preparation at maximum thermodynamic drug activity. The threshold concentration $c_{min_{eff}}$ has to be negligibly small compared with c_{max} to obtain accurate F values.

If the response, R, of standard and test preparations are determined at the same dose level and at the same time point the following equation results:

$$F = \frac{c_T - c_{min_{eff}}}{c_{ST} - c_{min_{eff}}} = \frac{R_T}{R_{ST}} \quad (9)$$

In addition to this approach, F may be determined from the horizontal distances between the dose–reponse curves of a standard and a test preparation at equal response levels:

$$\log F = \log D_{ST} - \log D_T \quad (10)$$

$$F = D_{ST}/D_T \quad (11)$$

In this case F is unaffected by $c_{min_{eff}}$ and thus equations 10 and 11 lead to more accurate values than equations 8 and 9. However, it again has to be mentioned that this approach applies only to zero-order penetration kinetics.

If the thickness, h, of the applied ointment preparations is kept constant, i.e. if V_V and A of all preparations are chosen to be the same during the experimental procedure, the resulting area and volume-standardized bioavailability factor F_h represents the ratio of the drug permeabilities, P_B, of a test and a standard preparation:

$$F_h = P_{B_T}/P_{B_{ST}} \quad (12)$$

With regard to the maximum response at equal dose levels below the drug solubility limit F_h may be written as:

$$F_h = \frac{\left(c_{max_T} - c_{min_{eff}}\right) \cdot Vd \cdot k_e \cdot h}{\left(c_{max_{ST}} - c_{min_{eff}}\right) \cdot Vd \cdot k_e \cdot h} = \frac{c_{max\ h_T} - c_{min_{eff}}}{c_{max\ h_{ST}} - c_{min_{eff}}} = \frac{R_{max\ h_T}}{R_{max\ h_{ST}}} \quad (13)$$

If the R_h values of standard and test preparations are determined at the same dose levels and time points, F_h equals:

$$F_h = \frac{c_{h_T} - c_{min_{eff}}}{c_{h_{ST}} - c_{min_{eff}}} = \frac{R_{h_T}}{R_{h_{ST}}} \quad (14)$$

Again, $c_{min_{eff}}$ has to be negligibly small compared with c_h and $c_{max\ h}$ to obtain correct estimations of F_h. More accurately, F_h may be determined from the horizontal distance between concentration–reponse curves at a certain response level:

$$\log F_h = \log c_{V_{ST}} - \log c_{V_T} \quad (15)$$

$$F_h = c_{V_{ST}}/C_{V_T} \quad (16)$$

where c_V is the drug concentration in the vehicle. Enhancement factors, EF, which are also called activity-standardized bioavailability factors F_a (Bach & Lippold 1998b), may be calculated by dividing the bioavailability factor F_h by the relative thermodynamic activity coefficient $\gamma_{T/ST}$. $\gamma_{T/ST}$ is defined as the ratio of the drug partition coefficients ST/reference phase and T/reference phase (Lippold & Reimann 1989a).

$$EF = F_h/\gamma_{T/ST} = J_{max_T}/J_{max_{ST}} \quad (17)$$

where J_{max} is the maximum drug flux. According to this equation enhancement factors are called true EF values throughout this paper.

In the absence of penetration enhancement and drug depletion from the ointment, F_h equals $\gamma_{T/ST}$.

In terms of the maximum response, EF may be defined as:

$$EF = \frac{\left(c_{max\ h_T} - c_{min_{eff}}\right) \cdot Vd \cdot k_e \cdot h \cdot c_{SV_T}}{\left(c_{max\ h_{ST}} - c_{min_{eff}}\right) \cdot Vd \cdot k_e \cdot h \cdot c_{SV_{ST}}} = \frac{c_{max\ h_T} - c_{min_{eff}}}{\left(c_{max\ h_{ST}} - c_{min_{eff}}\right) \cdot \gamma_{T/ST}} = \frac{R_{max\ h_T}}{R_{max\ h_{ST}} \cdot \gamma_{T/ST}} \quad (18)$$

where c_{SV} is the drug solubility in the vehicle.

According to equation 18, enhancement factors may be most accurately determined from the response plateau above the drug solubility limit in the vehicle. In analogy to equations 9 and 14, EF may also be calculated as:

$$EF = \frac{\left(c_{h_T} - c_{min_{eff}}\right)}{\left(c_{h_{ST}} - c_{min_{eff}}\right) \cdot \gamma_{T/ST}} = \frac{R_{h_T}}{R_{h_{ST}} \cdot \gamma_{T/ST}} \quad (19)$$

Enhancement factors may also be obtained from the horizontal distance between activity–reponse curves where the thermodynamic drug activity a is the ratio of the drug concentration in the vehicle and the drug solubility in this vehicle.

$$\log EF = \log a_{ST} - \log a_T \quad (20)$$

$$EF = a_{ST}/a_T \quad (21)$$

As in the case of dose– and concentration–reponse curves, the horizontal distance between activity–reponse curves is unaffected by $c_{min_{eff}}$.

Results and Discussion

In Figure 1 the effective drug concentration $c - c_{min_{eff}}$ (= R) at the receptor site is plotted vs time for various dose levels and a constant k_{p1} according to equation 1 for first-order input kinetics and equation 4 for zero-order input kinetics. It is

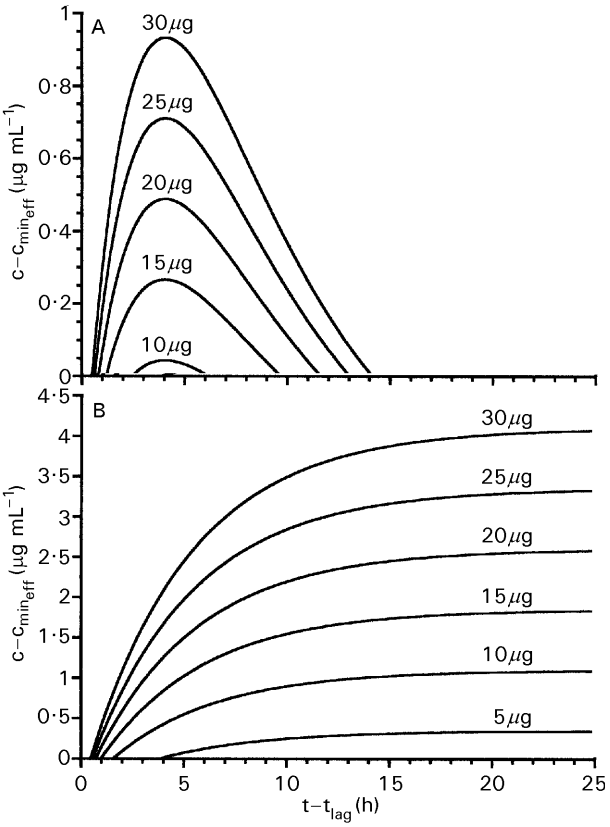

Figure 1. Effective drug concentration $c - c_{min_{eff}}$ (= pharmacodynamic response R) at the receptor site vs time profiles simulated for various dose levels with a k_{p1} value of $0{\cdot}3\ h^{-1}$. A. First-order input kinetics, B. zero-order input kinetics.

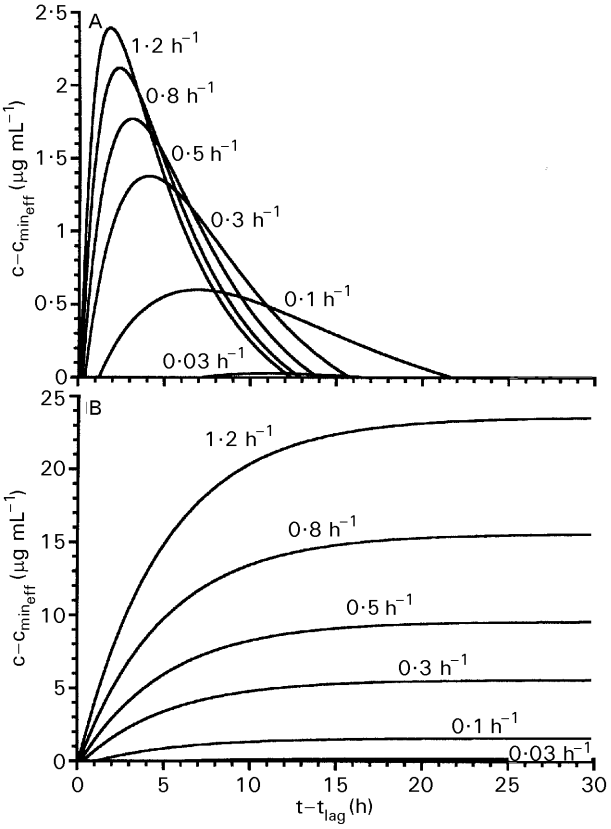

Figure 2. Effective drug concentration $c - c_{min_{eff}}$ (= pharmacodynamic response R) at the receptor site vs time simulated for various k_{p_1} values with a drug dose of 40 μg. A. First-order input kinetics, B. zero-order input kinetics.

obvious from these curves that the time points, t_{max}, at which c_{max} and thus $c_{max} - c_{min_{eff}}$ ($= R_{max}$) are reached are independent of the drug dose, no matter which penetration kinetic is looked at.

If concentration vs time profiles are simulated with various k_{p1} values at a constant dose level as shown in Figure 2, it becomes apparent that t_{max} is independent of k_{p1} for zero-order input kinetics only. With first-order penetration kinetics a non-linear decrease of t_{max} is observed with increasing k_{p1}, a relationship that is described by equation 3 and has to be taken into consideration if solution-type preparations are investigated. In general, c_{max} and R_{max} are reached earlier with first-order input kinetics and the obtained values are lower.

If R_{max} or R are determined at a defined time point with different solution-type preparations, i.e. different k_{p1} at various dose levels, dose–response curves may be generated for each preparation, provided that $c_{max} - c_{min_{eff}}$ and $c - c_{min_{eff}}$ correspond to R_{max} and R, respectively, and $c_{min_{eff}}$ is negligible compared with c_{max} and c. Dose–reponse curves with R_{max}, i.e. $c_{max} - c_{min_{eff}}$, as the parameter of response were calculated using equations 2 and 5 (Figure 3). Theoretically, a linear relationship exists between $c_{max} - c_{min_{eff}}$ (R_{max}) or $c - c_{min_{eff}}$ (R) and the applied drug dose (equations 1 and 2, and 4 and 5) provided that the drug solubility in the vehicle is not exceeded. This is true with both first- and zero-order penetration kinetics. Consequently, sigmoid curves are obtained if the response data are plotted against the logarithm of the dose, where the upper plateau is the result of the formation of drug suspensions at high dose levels. If vehicle effects are exclusively of thermodynamic nature resulting from different drug solubilities, curve profiles as shown in Figures 3A and 3B are obtained. As a result of the formation of drug suspensions at high dose levels, which leads to the maximum drug flux, all curves reach the same plateau level, no matter which penetration kinetic is applied. However, whereas in the case of zero-order input kinetics (Figure 3B) all curves are parallel to each other, with first-order kinetics this is true only for those portions of the curves that are below drug saturation in the vehicles. The horizontal distances between the curves are significantly lower with first-order penetration kinetics, an observation that may affect the deter-

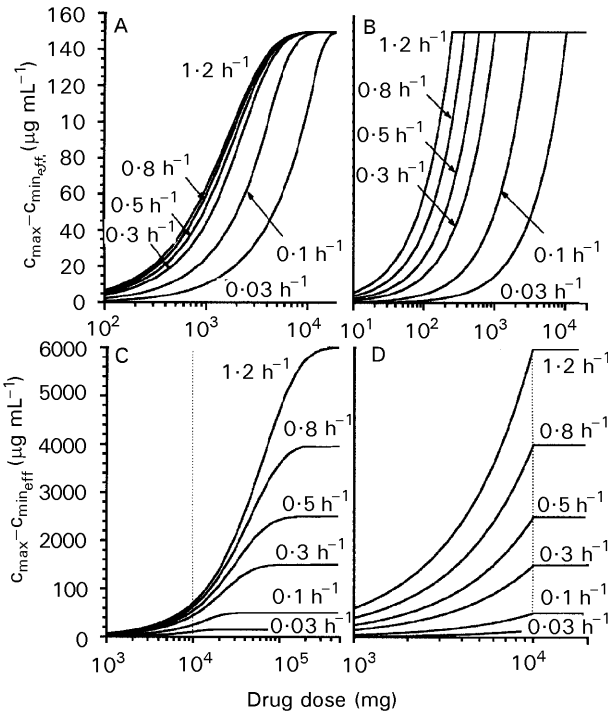

Figure 3. Dose–response curves ($c_{max} - c_{min_{eff}}$ = maximum response R_{max}) simulated for various k_{p1} values with first-order input kinetics (A and C) and zero-order input kinetics (B and D) under the assumption that differences in k_{p1} result from either thermodynamic (A and B) or penetration-enhancing (C and D) vehicle effects. The dotted line represents the drug solubility limit.

mination of bioavailability factors. The same applies to Figures 3C and 3D, where the dose–reponse curves are the result of penetration-enhancing vehicles with a constant drug solubility of 10 mg mL^{-1}. It is obvious from the curves, that with first-order input kinetics (Figure 3C) the upper response plateaus are reached at dose levels that exceed the drug solubility limit. The amount of undissolved drug in the vehicles required to reach the response plateau increases with increasing k_{p1}. The curve profiles above the solubility limit in Figure 3C describe the conversion from first-order to zero-order kinetics. With dose levels that lead to response values in the plateau region zero-order input kinetics are obtained.

As in Figure 3 the horizontal distances between the curves do not depend on the response level at which they are determined provided that the drug solubility level is not exceeded, relative bioavailability factors may be obtained from any portion of the curves as long as it is below the drug solubility limit. Another method to determine relative bioavailability factors is the calculation of R_{max} or R ratios as described by equations 8 and 9. The two methods only lead to the same value when $c_{min_{eff}}$ is negligible when compared with c_{max} or c. This is because the ratios of the drug concentrations in the receptor compartment obtained with a test and a standard formulation at equal dose levels correspond to the dose ratios between a standard and a test preparation (equations 2 and 5).

Bioavailability factors may be calculated accurately according to equations 8, 9 and 11, and 13, 14 and 16 only if zero-order penetration kinetics are given. This is a result of the linear relationship between c_{max} or c and k_{p1} under infinite dose conditions. Table 1 gives an overview of bioavailability factors determined for first-order and zero-order input kinetics from the dose–reponse curves in Figure 3 using equation 11, including the true values calculated according to equation 7. Whereas bioavailability factors obtained with zero-order input kinetics correspond to the true values in the applied mathematical model, first-order kinetics lead to a significant underestimation of the relative bioavailability if a standard preparation with a small k_{p1} is used. Accordingly, a standard with a high k_{p1} leads to an overestimation of the bioavailability factors.

Table 1. Bioavailability factors (F) determined for first-order and zero-order input kinetics from the dose–response curves in Figure 3 using equation 7 (true values) and equation 11 (D ratios below drug saturation).

k_{p1} (h^{-1})	F (true)	F (D ratios)	
		First-order	Zero-order
0·03 (standard)	1	1	1
0·1	3·33	2·329	3·33
0·3	10	4·141	10
0·5	16·66	5·058	16·66
0·8	26·66	5·87	26·66
1·2	40	6·511	40

For concentration–reponse curves, as opposed to dose–reponse curves, it has to be taken into consideration that V_V is included in k_{p1} and that a linear relationship between $c_{max} - c_{min_{eff}}$ and the drug concentration in a vehicle can only be expected mathematically with zero-order penetration kinetics (equation 5). However, if V_V is kept constant and all preparations are applied at the same volume, a change in the drug concentration means a change only in the drug dose and in this case no difference exists between concentration–reponse and dose–reponse curves with first-order penetration kinetics. In other words, in this case F corresponds to F_h. It is important to consider this theoretical fact because in practice, dose–reponse curves do not play an important role in bioavailability studies involving transdermal preparations compared with concentration–reponse curves.

To distinguish between vehicle effects caused by different thermodynamic drug activities and those resulting from penetration-enhancing effects, the enhancement factor EF, which is also called enhancement ratio (Goodman & Barry 1988) or activity-standardized bioavailability factor (Bach & Lippold 1998a), has been introduced (Kadir et al 1988; Leopold & Lippold 1995). EF may be obtained either by dividing the factor F_h determined from concentration–reponse curves by the relative thermodynamic activity coefficient $\gamma_{T/ST}$ according to equation 17 or in analogy to the determination of F and F_h from activity–reponse curves. To transform concentration–reponse into activity–reponse curves the drug concentration of each ointment preparation has to be divided by the drug solubility in the respective ointment base. In the absence of enhancement effects the resulting activity–reponse curves of standard and test preparations should be superimposable. Any distance between standard and test curves indicates a barrier-modifying action of the ointment base. In Figure 4, activity–reponse curves, transformed from concentration–reponse curves, are shown for both first- and zero-order kinetics under the assumption that differences in k_{p1} result from either thermodynamic (Figures 4A, 4B) or penetration-enhancing (Figures 4C, 4D) vehicle effects. An activity value of 1 indicates that the solubility limit of the drug in the vehicle has been reached, a fact that leads to the maximum

response, i.e. a response plateau, only in the case of infinite dose conditions. Under finite dose conditions these plateaus are reached as soon as a sufficient amount of undissolved drug is present in the vehicle that guarantees zero-order order penetration kinetics over an extended time period. The curves are superimposable with zero-order penetration kinetics only (Figure 4B). The solubility in the vehicle is included in k_{p1} as the denominator of the partition coefficient $PC_{B/V}$, therefore the observed deviations of the test curves from the standard curve always have to be expected with first-order penetration kinetics (equation 2). The shift of the test curves to higher drug activities lead to the wrong impression that the test vehicles have penetration retarding properties, i.e. the enhancement factors are underestimated in the case of a standard with a low constant k_{p1}. Accordingly, an overestimation of the enhancement factors occurs if standard preparations with high constant k_{p1} are chosen. Table 2 gives an overview of the true enhancement factors (equation 17) compared with those obtained from the activity–reponse curves in Figure 4 with first- and zero-order input kinetics (equations 18 and 21), respectively. The calculations were made under the assumption that differences in k_{p1} are the result of either thermodynamic or penetration-enhancing vehicle effects. Only with zero-order input kinetics are enhancement factors corresponding to the true values obtained, a fact that manifests itself in superimposed activity–reponse curves. First-order kinetics generally lead to a misestimation of enhancement factors, even if no penetration enhancement occurs and enhancement factors of unity should result. If enhancement factors are calculated as response ratios from the respective response plateaus after application of drug suspensions, the resulting data are reasonably accurate, although they are affected by the minimum effective threshold concentration $c_{min_{eff}}$.

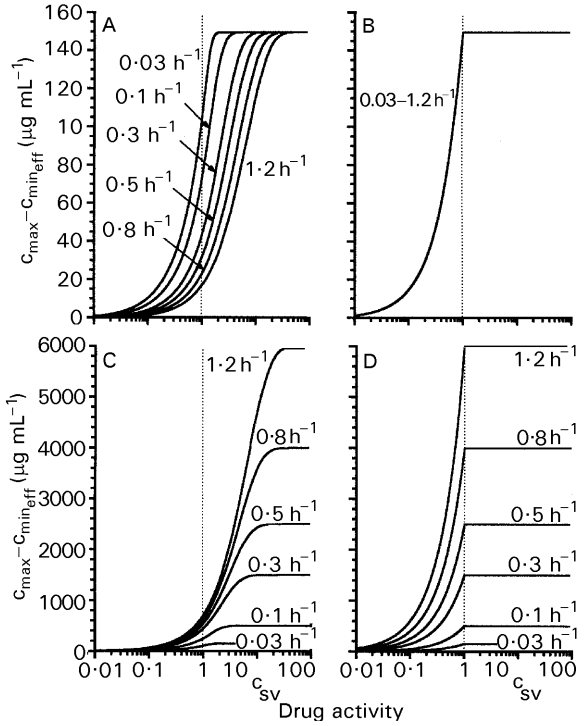

Figure 4. Activity–response curves ($c_{max} - c_{min_{eff}} =$ maximum response R_{max}) simulated for various kp_1 with first-order input kinetics (A and C) and zero-order input kinetics (B and D) under the assumption that differences in kp_1 result from either thermodynamic (A and B) or penetration-enhancing (C and D) vehicle effects. The dotted line represents the drug solubility limit. c_{SV} is the drug solubility in the vehicle.

Penetration enhancement is the result of an increase of the drug solubility and/or the drug diffusion coefficient in the barrier. As the drug diffusion coefficient is inversely proportional to the

Table 2. Enhancement factors (EF) determined for first-order and zero-order input kinetics from the activity–response curves in Figure 4 using equation 17 (true values), equation 18 (R_{max} ratios determined from response plateaus) and equation 21 (a ratios below drug saturation), and under the assumption that differences in k_{p1} result from either thermodynamic or penetration-enhancing vehicle effects.

K_{p1} (h^{-1})	EF (true)		EF (R_{max} ratios)		EF (a ratios)			
					First-order		Zero-order	
	Thermo-dynamic	Penetration-enhancing	Thermo-dynamic	Penetration-enhancing	Thermo-dynamic	Penetration-enhancing	Thermo-dynamic	Penetration-enhancing
0·03 (standard)	1	1	1	1	1	1	1	1
0·1	1	3·33	1	3·34	0·699	2·329	1	3·33
0·3	1	10	1	10·024	0·414	4·141	1	10
0·5	1	16·66	1	16·709	0·304	5·058	1	16·66
0·8	1	26·66	1	26·735	0·220	5·87	1	26·66
1·2	1	40	1	40·104	0·163	6·511	1	40

lag time of drug penetration (Crank 1956; Flynn & Roseman 1971), the time point t_{max} until the maximum drug concentration in the receptor compartment is reached will be affected by a decrease or increase of the drug diffusion coefficient. In the case of penetration enhancement a decrease in t_{lag} and thus t_{max} has to be expected. If zero-order input kinetics are given, the determination of relative bioavailability and enhancement factors is mainly affected by an increase in drug diffusion if the factors are calculated using equations 9, 14 or 19. Depending on the chosen standard preparation F, F_h and EF values will either be over- or underestimated because in the case of an increased drug diffusion coefficient c and $c - c_{min_{eff}}$ increase faster than expected (equation 4). The threshold concentration in the receptor compartment $c_{min_{eff}}$ can have a significant influence on the calculation of F, F_h and EF from equations 8 and 9, 13 and 14, and 18 and 19. Therefore, this approach is generally not recommended for estimation of bioavailability and penetration enhancement factors, although from an experimental point of view it represents the most timesaving method.

The decrease in t_{lag} also affects the determination of F, F_h and EF if drug penetration follows first-order penetration kinetics. The decrease in t_{max} with increasing k_{p1} and decreasing t_{lag} is described by equation 3. As an increase of the diffusion coefficient in the barrier caused by the action of penetration enhancers on the stratum corneum lipids leads to both an increase in k_{p1} and a decrease of t_{lag}, an even more rapid decrease in t_{max} may be observed.

If the simulated pharmacokinetic data are compared with the results of pharmacodynamic measurements of the maximum response, several unexpected observations made in the past may be explained by applying the theories presented. For instance, measurements of the pharmacodynamic response after application of solution-type benzyl nicotinate ointment preparations resulted in a significant difference between the bioavailability factors F_h determined from the horizontal distance between concentration–response curves with the maximum skin temperature as response parameter, and values determined with the response parameter 1/lag time of onset (Lippold & Teubner 1981b). As the lag time of onset data lead to reasonably accurate F and F_h values even if drug depletion occurs, as in the case of first-order input kinetics (Leopold 1998a), the observed deviations from the true F_h may be attributed to the misestimation of the relative bioavailability as a result of first-order penetration kinetics (Table 1, Figure 3A). Moreover, the shift of the time point of maximum response t_{max} with varying k_{p1} and the determination of R_{max} in the case of high k_{p1} values is critical from an experimental point of view and can lead to additional bias. Infinite dose conditions allow the measurement of the intensity of a response at any given time point, provided that the diffusion coefficient is not affected significantly by the applied preparations.

Another common bioassay where the maximum pharmacodynamic response is measured is the vasoconstrictor or skin blanching assay used for evaluation of topical corticosteroid formulations (McKenzie & Stoughton 1962; Barry & Woodford 1978; Lippold & Schneemann 1984; FDA 1995; Bach & Lippold 1998a). As corticosteroids are known to form a drug reservoir in the stratum corneum, the presented pharmacokinetic model (one compartment) does not sufficiently describe the true kinetic conditions (Lippold & Schneemann 1984). In this case, simulations based on a two-compartment model, with the stratum corneum representing an additional compartment, would have provided more realistic kinetic data (Naito & Tsai 1981; Lippold & Schneemann 1984). However, with regard to the determination of the relative bioavailability and penetration enhancement from concentration– and activity–reponse curves, respectively, the general postulations made above for the one-compartment model also apply to the two-compartment model. Consequently, in the case of the vasoconstrictor assay a misestimation of the relative bioavailability or penetration enhancement factors has to be expected with first-order input kinetics. Such a misestimation is probably one of the reasons for the significant difference between the enhancement factors obtained from the horizontal distance between activity–reponse curves and those calculated according to equation 18 after application of suspension-type preparations (Bach 1995; Bach & Lippold 1998a). The study dealt with the quantification of penetration enhancement of lipophilic penetration enhancers using the vasoconstrictor assay according to the FDA Guidance (FDA 1995) in which the enhancers served as vehicles for the model corticosteroid betamethasone-17-benzoate. An inert vehicle with low drug solubility resulting in a high k_{p1} was chosen as standard. It was found that enhancement factors determined as the relative maximum skin blanching intensity after application of drug suspensions (infinite dose conditions) amounted to between 1 and 1·5, whereas factors determined from the horizontal distance between activity–reponse curves obtained with drug solutions (finite dose conditions) reached values of up to 6·4. If a vehicle with a high drug solubility resulting in a low k_{p1} had

been chosen as standard vehicle, an underestimation of the enhancement factors would have been observed as expected from the results of the presented simulations (Table 2).

In order to obtain the most accurate values from pharmacodynamic measurements of the maximum response the following recommendations should be considered. It is essential to guarantee zero-order penetration kinetics. This can be achieved by application of large ointment volumes ensuring infinite dose conditions. The response should not be determined at a time point earlier than t_{max}. If suspension-type ointments with a sufficient amount of undissolved drug are used to obtain zero-order penetration kinetics, it has to be remembered that the maximum drug flux is achieved, which may in some cases lead to saturation of the receptor binding sites making it impossible to distinguish between different preparations. Application of suspension-type preparations allows the determination of enhancement factors as the ratio of the maximum response values obtained with a test and a standard preparation, however, although a time-saving method, it can be significantly influenced by the minimum threshold concentration at the receptor site.

Although time-consuming, bioavailability and penetration enhancement data should always be determined from the horizontal distance between concentration– and activity–reponse curves, respectively, because these data are not affected by the minimum threshold concentration at the receptor site.

To distinguish between thermodynamic effects resulting from different drug solubilities in the ointment bases and true penetration enhancing properties, i.e. barrier-modifying effects, drug concentrations in the ointment bases should be adjusted to equal thermodynamic drug activities. In this way, activity–reponse curves may be obtained instead of dose– or concentration–reponse curves allowing a determination of enhancement factors.

Bioavailability and enhancement factors should not be determined by calculation of the ratio of the maximum response or the response at a given time point of a test and a standard preparation. This is because the influence of the minimum threshold concentration at the receptor site on the data cannot easily be predicted.

The presented pharmacokinetic simulations cannot be applied quantitatively to the in-vivo conditions of a pharmacodynamic study dealing with the measurement of the maximum response because of the assumptions and simplifications made. However, they provide information on how to set up the experiments and how to determine accurately bioavailability and penetration enhancement data from dose–response curves.

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