Pharmacodynamic Measurements of Methyl Nicotinate Percutaneous Absorption

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Abstract: The local kinetics of percutaneous absorption provide information of relevance to the treatment of skin diseases and to the potential efficacy of transdermally delivered chemotherapy for systemic effect. This paper describes two non-invasive procedures (laser Doppler velocimetry and photopulse plethysmography) which permit pharmacodynamic measurements of methyl nicotinate skin penetration to be made *in vivo* in man. The methods are sensitive to the local vasodilative action elicited by the nicotinic acid ester. Dose-response behavior as a function of time has been monitored (1) over the concentration range 5–100 mM, and (2) by variation of drug application time and administration area. At the higher concentrations used, the magnitude of the erythemal response is saturable, and the effect is then progressively prolonged by further increasing the applied dose. Analysis of the data permits assessment of (a) the kinetics of drug delivery to and depletion from the site of action, and (b) the hypothetical level of steady state drug input necessary to sustain 50 % of the maximum detected response. Measurements of the type described here may prove useful, therefore, for elucidating otherwise inaccessible aspects of transcutaneous kinetics *in vivo*.

Introduction

The transport of molecules across the skin of man is a subject currently experiencing a resurgence of interest. A major reason for the activity in this area is the recognition that transdermal delivery of drugs for systemic effect is possible and attractive in several ways (1–4). The use of the skin as a port of entry into the body, though, is far from trouble free. Indeed, it is no exaggeration to say that, in the past, the skin has been considered too good a barrier to be feasible for systemic drug delivery. In addition, the process of skin penetration is complex; movement from the surface into the tissue is a multistep phenomenon involving a variety of transport, partitioning and other events (5). Various experimental approaches to study percutaneous absorption are needed because of the complicated nature of the absorption process.

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Percutaneous absorption studies with human skin can be broadly classified into two groups: (a) *in vivo* experiments, in which absorption is quantified somewhat indirectly by drug measurement in blood or urine, and (b) *in vitro* experiments performed in glass diffusion cells which allow assessment of drug penetration through excised skin. The latter experiments naturally provide valuable information pertinent to the interpretation and prediction of the former, yet, they suffer the criticism that the laboratory system can only approximate the *in vivo* situation. The problem in terms of quantifying the local time-course of percutaneous absorption *in situ* in man, is assay of penetrant arrival at the local elimination site, namely the dermal vasculature.

Experiments are described here which attempt to follow the local absorption events *in vivo*. The penetration of a potent topical vasodilator (the methyl ester of nicotinic acid (6)) is assessed by two techniques sensitive to alterations in cutaneous microvasculature perfusion. The chemical penetrates the stratum corneum, reaches the dermal capillaries and exerts its pharmacological action. Because only limited amounts of methyl nicotinate are applied, the vaso-response passes through a maximum and then diminishes to zero, consistent with the depletion of agent at the active site (i. e., uptake into the capillaries and systemic dilution). It is the time course of these pharmacodynamic events which the methodology monitors.

The techniques used are laser Doppler velocimetry (LDV) and photopulse plethysmography (PPG). They operate on different optical principles yet are both totally non-invasive – the relevant experimental information being collected via small probes held to the skin surface by adhesive tape. LDV uses a laser light source and the Doppler effect to generate an output proportional to the flux of blood through the microcirculation under observation (7–10). PPG, on the other hand, essentially evaluates the local blood volume as determined by hemoglobin's absorbence of an infra-red light source (11). Preliminary indication that LDV and PPG are sensitive to the absorption of methyl nicotinate (MN) was reported recently (12). The data showed good correlation with the onset of visible erythema (subjectively assessed) caused by this chemical (6). Subsequently, PPG was used to measure the pharmacodynamic response to methyl nicotinate at five different application sites (13). Only one concentration of vasodilator was studied and saturation of the effect was apparent. This investigation focusses attention upon one anatomic application site (the forearm) and addresses the dose-response behavior of

methyl nicotinate as assessed by both LDV and PPG. Thus, the ultimate objective of the present study was to test whether the kinetic processes of local skin absorption and elimination can be determined using the pharmacodynamic effects of methyl nicotinate on the cutaneous microcirculation.

Materials and Methods

1. Laser Doppler Velocimetry (LDV).
The technique has been described in detail elsewhere (7–10). Briefly, light at 632.9 nm from a 5 mW He-Ne laser is directed into the skin. The radiation is multiply-scattered and reflected by both stationary tissue components and mobile red blood cells. The former back-scatter radiation at the incident frequency, the latter reflect light which has been frequency (or Doppler)-shifted by an amount proportional to the product of their number multiplied by their velocity. The capillary perfusion monitor (LD 5000, Medpacific Inc., Seattle, Washington) employed separates the frequency-shifted component, amplifies the signal and displays the result as a fluctuating voltage. Laser light is transmitted to the skin from the capillary perfusion monitor, and the reflected radiation is returned along identical optical fibers. Physical support to the fibers at the point where they interface with the skin is provided by a small cylindrical probe (1.9 cm diameter, 1.5 cm high) which is attached to the application site using a double-sided adhesive disc.

2. Photopulse Plethysmography (PPG).
Descriptions of this procedure have appeared previously (8, 13). An infra-red light emitting diode directs radiation into the skin. The wavelength range of the source (800–940 nm) spans a region for which the tissue is transparent; on the other hand, hemoglobin absorbs strongly in this part of the spectrum and hence the reflected radiation, which is measured with a phototransistor, contains information about the volume of blood in the skin site under observation. As with LDV, PPG is believed to monitor the microcirculation to a depth of 1–2 mm (12, 13). Again, radiation is transmitted to the skin and received back via a small (2 cm long x 1 cm wide x 1 cm deep) probe housing the diode and phototransistor, which is held in position by double-sided adhesive tape. Processing of the blood volume fluctuations is performed by a photoplethysmograph (Medasonics, Mountain View, California). Increases in perfusion are registered by an enhancement of the control output pulsations (8, 12, 13).

3. Experimental.
Dose-response behavior following topical application of the potent vasodilator methyl nicotinate (MN) (Sigma Chemical Co., St. Louis, Missouri) has been measured. Concentrations in the range 5–100 mM, prepared directly in distilled water, were used; the pH of these solutions was 5.5 ± 0.5. The chemical was applied via a saturated test patch (1 cm diameter) (Al-test, Imeco-ab, Södertälje, Sweden) to prevent solution from running and spreading over the skin surface; the volume of solution held by the circular patch was 50 µl (± 2 %). The application site was the ventral surface of the forearm on the midline at the boundary between the upper and middle thirds. A baseline perfusion measurement (as assessed by either LDV or PPG) was taken at the application site and the instrument probe removed; prior to these measurements the capillary perfusion monitor was zeroed using its internal circuitry, and the plethysmograph was calibrated with an oscillating reflection device, which provides a constant signal to standardize the PPG sensor's sensitivity.

Dose-response behavior was also investigated by changing two alternative variables: (a) the application area, and (b) the application time. In the first case, results using the standard patch were compared with those using a 0.6 cm diameter patch. Methyl nicotinate concentration was held constant at 100 mM, and the application time continued to be 15 seconds. In the second instance, maintaining methyl nicotinate concentration at 100 mM and using the 1 cm diameter patch only, application times of 5, 15 and 30 seconds were employed and the results compared.

The experimental subjects were four young (20–30 years), healthy adults, three males and one female. The experiments were performed in a single well-ventilated room under constant temperature and humidity conditions (T = 23 ± 2°C, RH = 50–70 %). In the first part of the study each concentration was tested for each subject on at least three separate occasions. Using bilateral sites on both arms, LDV and PPG data were collected simultaneously. Repeat measurements in each individual subject were performed at least 3 days apart.

Results

LDV and PPG reponse curves as a function of time for the four subjects receiving four different methyl nicotinate concentrations topically as described above are shown in Figs. 1 and 2, respectively. The results are summarized in Table I. Three parameters have been selected to describe the time course and extent of the pharmacodynamic response: (*1*) the magnitude of the maximum response; (*2*) the area under the response-time curve; and (*3*) the time required for the response to return to 75 % of the maximum value.

Saturability of the local microvasculature response was consistently observed. In Fig. 3, this phenomenon is illustrated by a plot of normalized maximum response versus concentration. Alternatively, Fig. 4 shows the concentration dependence of the area under the response-time curve. Both Figs. 3 and 4 have characteristics of saturable dose-response behavior (14).

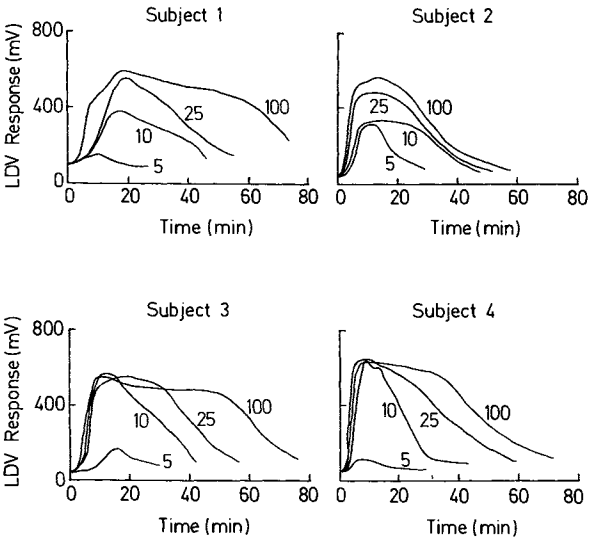

Fig. 1 LDV response curves as a function of time and methyl nicotinate concentration for four subjects. The curves corresponding to the various drug concentrations (in mM) are indicated on the graphs. Each curve shown is the mean of at least 3 separate determinations. Variation about the means was not greater than ± 10 %.

Finally, Table II shows typical results when the area of methyl nicotinate application is altered and indicates the sensitivity of cutaneous perfusion changes to the duration of application.

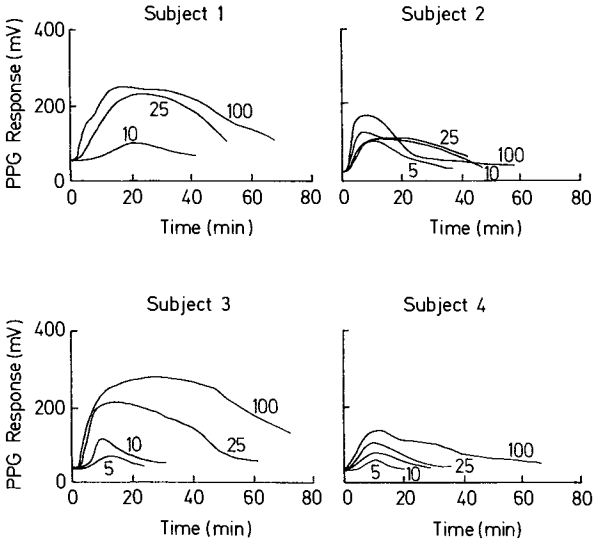

Fig. 2 PPG response curves as a function of time and methyl nicotinate concentration for four subjects. The curves corresponding to the various drug concentrations (in mM) are indicated on the graphs. Each curve shown is the mean of at least 3 separate determinations. Variation about the means was not greater than ± 10 %.

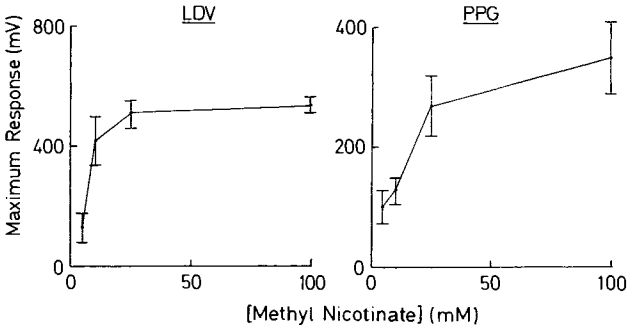

Fig. 3 Maximum instrument response (± S.E.) as a function of applied methyl nicotinate concentration (see Table I). Dependence as assessed by both LDV and PPG is shown.

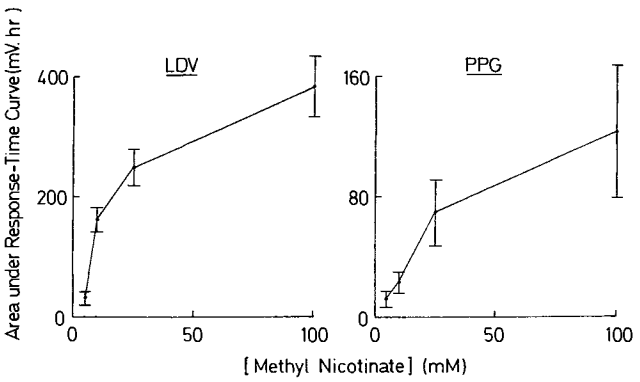

Fig. 4 Area under the response-time curve (± S.E.) as a function of applied methyl nicotinate concentration (see Table I). Dependence as assessed by both LDV and PPG is shown.

Discussion

Both LDV and PPG permit objective observation of the onset, duration and decay of the local pharmacologic response to the vasodilating stimulus. The results in Figs. 1–4 and in Table I suggest that for the subject population studied, topical application of methyl nicotinate in the manner described, at concentrations in excess of 10–25 mM, results in saturation of the pharmacodynamic effect. Despite the simple aqueous vehicle and the short period of skin contact, sufficient chemical does penetrate the cutaneous barrier to perturb significantly the local microvasculature.

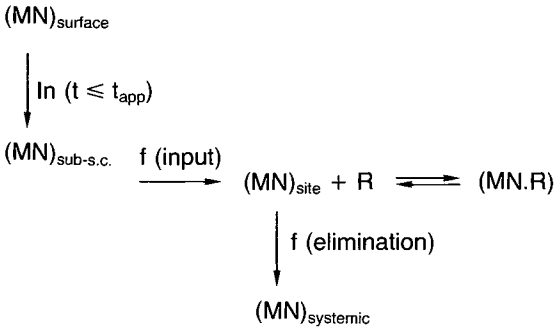

Fig. 5 Schematic representation of the kinetic events observed in the experiments of this study (s. c. = stratum corneum).

Table I. Summary of Dose-Response Data from Four Subjects[a]. (MN: methyl nicotinate).

[MN](mM)	Maximum instrument response (mV)		Area under response-time curve (mV · hr)		Time (min) for response to decay to 75 % of maximum value	
	LDV	PPG	LDV	PPG	LDV	PPG
100	536 ± 23	173 ± 30	385 ± 52	124 ± 44	59 ± 7	56 ± 11
25	507 ± 39	135 ± 26	252 ± 30	70 ± 22	44 ± 3	41 ± 7
10	421 ± 78	66 ± 11	163 ± 20	23 ± 7	36 ± 8	31 ± 5
5	131 ± 52	48 ± 14	32 ± 13	12 ± 5	21 ± 4	20 ± 2

[a] The information has been extracted from Figs. 1 and 2 and each value quoted is the mean ± the standard error of the mean. The results have been normalized to eliminate difference in control (i. e., pre-drug application) perfusion levels.

Table II. Dose-Response Data as a Function of Application Time and Application Area[b].

Application area (mm^2)	Application time (sec)	Maximum instrument response (mV)		Area under response-time curve (mV · hr)		Time (min) for response to decay to 75 % of maximum value	
		LDV	PPG	LDV	PPG	LDV	PPG
28.3	15	409	86	274	41	50	38
78.5	15	496	148	234	43	40	25
78.5	5	246	68	188	23	56	34
78.5	15	496	148	250	49	40	25
78.5	30	517	124	462	88	63	57

[b] The results have been normalized to eliminate differences in control (i. e., pre-drug application) perfusion levels.

A primary aim of this investigation is to attempt to quantify certain aspects of transcutaneous kinetics *in vivo* in man by direct measurement of the local penetration events. As a reasonable starting point, the rate processes taking place may be simplistically formulated as shown in Fig. 5. This representation contains a number of assumptions. Firstly, delivery of methyl nicotinate across the stratum corneum is constant (In) as long as the patch is present. Next, drug then diffuses to its vasoactive site by a process that can be represented by a single simple input function

$$f(\text{input}) \equiv k_i, \tag{1}$$

where k_i is a first-order rate constant (15). Thirdly, the loss from the vasoactive site via capillary uptake of drug by the dermal microcirculation is a simple elimination function, which current experimental evidence (16) suggests is also first-order

$$f(\text{elimination}) \equiv k_e . \tag{2}$$

Finally, it is postulated that at the capillary wall methyl nicotinate binds to a receptor site (in an unknown stoichiometry) producing a complex, the concentration of which is related to the magnitude of the observed pharmacological effect by the Hill equation (17)

$$(E-E_o) = \frac{E_{max} \times C^N}{C_{50}^N + C^N}, \tag{3}$$

where C is concentration at the receptor, E is measured response, E_o is the baseline response (i. e., perfusion level), E_{max} is the maximum response, C_{50} is the concentration producing 50 % of E_{max}, and N is a parameter affecting the steepness of the E versus C curve.

The experiments that have been performed produce data which show how E varies with time for different topically applied concentrations of methyl nicotinate. The concentration of drug at the site of action will be controlled by the input and elimination kinetics k_i and k_e, and a simple biexponentional equation can be derived (18) which describes this dependence. However, as will be shown below, in many instances the results do not allow k_i and k_e to be differentiated because their values are similar. One may proceed, none the less, by setting

$$k_i = k_e = k . \tag{4}$$

By doing so, it is possible to write an equation for the normalized concentration (u) of methyl nicotinate within the skin (i. e., sub-stratum corneum):

$$u = kt \exp(-kt), \tag{5}$$

where

$$u = \frac{([MN] \text{ in skin}) \times (\text{local volume of distribution, V})}{A \cdot C_{APP} \cdot P \cdot t_{APP}} \tag{6}$$

C_{APP} is the applied methyl nicotinate concentration in the patch, A is the patch area and t_{APP} is the duration of patch application (0.25 minutes). P describes the stratum corneum permeability to methyl nicotinate and may be expressed by Eq. 7

$$P = \frac{D \cdot K}{h}. \tag{7}$$

D is therefore the diffusion coefficient of methyl nicotinate through stratum corneum of thickness h, and K is the stratum corneum/water partition coefficient of the drug. It follows that the denominator of Eq. 6 is the amount (Q) of drug which is delivered sub-stratum corneum by a patch application of duration t_{APP}. This formulation assumes that the total drug input ($A \cdot C_{APP} \cdot P \cdot t_{APP} = Q$) occurs instantaneously at t=0, rather than over 15 seconds (t_{APP}). This is a reasonable approximation given the tempo of subsequent observed events (see Figs. 1 and 2).

Since a noticeable time-lag (t_L) between drug application and response onset was observed (see Figs. 1 and 2), Eq. 5 is more appropriately given by

$$u = kt' \exp(-kt'), \tag{8}$$

where $t' = t - t_L$. The time-lag serves in an empirical fashion to compensate for the inadequacies of the input model as formulated above.

Finally Eqs. 3 and 8 are combined in an unified expression which links measured effect, time and C_{APP}. The concentration terms (C) in Eq. 3 are given by

$$C = \frac{uQ}{V}, \tag{9}$$

but further work must be done to express C_{50} in terms of C_{APP}. Consider a patch applied for an indefinite time and that the concentration of methyl nicotinate within the patch over this period is maintained constant at C_{SS}. Then the steady-state concentration at the vasoactive site would, if all previous assumptions hold, be C_{SS}.A.P/(V.k.). Hence, $C_{SS,50}$ would correspond to C_{50} in Eq. 3. Parameterizing Eq. 3 in this way, and eliminating the common terms (A, P, V) in numerator and denominator, yields

$$(E-E_o) = \frac{E_{max} \times R^N}{R^N + C^N_{SS,\,50}}, \qquad (10)$$

where

$$R = k \cdot C_{APP} \cdot t_{APP} \cdot u. \qquad (11)$$

The reduction has value, not only for linking (in Eq. 10) E, C_{APP} and t', but also for allowing the potential for estimating the $C_{SS,50}$ parameter. In terms of the derivation elaborated above, $C_{SS,50}$ effectively estimates the hypothetical steady-state patch concentration of methyl nicotinate which would be necessary to maintain half the possible maximum increase in skin blood flow that the chemical can produce (assuming no tolerance). Thus, $C_{SS,50}$ may be considered a measure of the sensitivity of the cutaneous microcirculation to drug-induced vasodilatation.

Eq. 10 was fit to the experimental data using a nonlinear least squares regression program (19). It was possible to identify parameter values for k, $C_{SS,50}$, E_{max} and N for each set of results. To reduce the number of parameters being fitted at any one time, C_{APP}, E_o and t_L were fixed; C_{APP} for obvious reasons, E_o and t_L because they can be directly obtained from the data. The outcome of this analysis is summarized in Table III. Average parameter estimates (from data at 4 concentrations) for all subjects using both techniques are found and then, using these means and their standard errors, a "population" average is calculated. These averages are weighted means in which both the standard error of the parameter estimates and the biological variation in parameters are correctly combined through maximum likelihood estimation. The procedure is briefly described elsewhere (19).

The fits to the data generated by the regression analysis are very good. Fig. 6 exemplifies this fact and shows the computer fits to the LDV data of subject 2. Attempts were also made to analyse the results with the biexponential equation which describes u (Eq. 5) when $k_i \neq k_e$:

$$u = \frac{k_i}{k_i - k_e}[\exp(-k_e t) - \exp(-k_i t)] \qquad (12)$$

Although this proved successful on certain occasions, albeit with high standard errors for the kinetic constants, frequently the regression program forced the rate parameters towards the same value and consequently failed because of the effect of the denominator in Eq. 12. It seems reasonable to expect that k_i and k_e will be close if, indeed, they both describe intra-dermal (i.e., sub-stratum corneum) diffusion processes.

The results in Table III provide an indication both of the intra- and inter-subject variability and of the very acceptable agreement between measurements made using LDV and PPG. Of particular interpretive interest are the $C_{SS,50}$ and k parameters. The former indicates, as has been observed, that a range of sensitivities to the vasodilatation stimulus may be expected. It is important to point out, however, that $C_{SS,50}$ values estimated from the lowest concentration data are imprecise because these results provide little or no indication of the level at which response saturation occurs. Thus, future experiments must include studying a larger sample of subjects to whom high doses are administered. In this way more consistently reliable estimates of $C_{SS,50}$ will be acquired.

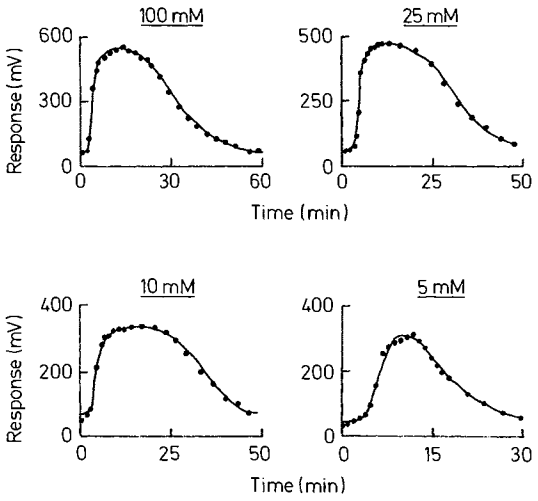

Fig. 6 Non-linear least squares regression analysis and fit using Eq. 10 (continuous line) to the LDV experimental data (solid circles) of subject 2.

Table III. Results of Nonlinear Least Squares Regression Analysis of the Data Using Eq. 10. Average Values ± Standard Errors are given. The Population Averages were Obtained Using Appropriately Weighted Individuals Subject Parameters (See Text).

Subject	k(min^{-1})		$C_{SS,50}$(mM)		E_{max}(mV)		N	
	LDV	PPG	LDV	PPG	LDV	PPG	LDV	PPG
1	0.063	0.046	0.19	0.15	524	313	2.78	3.31
	±.007	±.001	±.01	±.06	±38	± 39	±.39	±.68
2	0.117	0.143	0.18	0.04	441	110	2.24	1.38
	±.010	±.026	±.10	±.01	±65	± 3	±.34	±.44
3	0.082	0.075	0.09	0.12	517	262	2.51	1.75
	±.008	±.010	±.01	±.03	±44	± 15	±.43	±.16
4	0.167	0.086	0.09	0.08	642	503	1.19	3.05
	±.010	±.006	±.02	±.05	±82	±644	±.19	±.88
Population	0.106	0.079	0.12	0.08	521	224	2.10	1.94
	±.020	±.010	±.03	±.02	±25	± 50	±.33	±.25

The k values indicate a half-life for methyl nicotinate delivery to and elimination from the site of action on the order of 8 minutes (shortest t ½ = 4 minutes (LDV data of subject 4), longest t ½ = 15 minutes (PPG data of subject 1)). This result is consistent with our previous non-invasive observations for this vasodilator (12, 13) and with other reports for different substances (21–23). The value is also in excellent agreement with a recent investigation (24), which studied the radial diffusion of methyl nicotinate in the dermis by following the expansion of erythematous area caused after topical application of the chemical. These workers analyzed their results with a radial transport model which included capillary uptake and, under certain conditions, re-equilibration with the local dermal tissue. They showed that the lifetime of methyl nicotinate in the dermis was 3–10 minutes.

However, it must be stated that, although the techniques used in these earlier determinations were not equivalent, either the chemical studied or the means of delivery (e. g., intradermal injection) to the elimination site resulted in local vasodilatation. The commonality among k_e values must therefore be viewed circumspectly, and k for a vasoconstrictor, for example, cannot be confidently predicted. It remains to be seen whether the noninvasive methodology used here can successfully monitor vasoconstrictor transdermal kinetics/local pharmacodynamics.

Regarding methyl nicotinate delivery across the stratum corneum, it appears to be rapid, but there is a delay between drug application and the onset of increasing microvasculature perfusion. Although we have used a time-lag to model this delay, any relationship between t_L here and a classic diffusional lag-time (25, 26) is not established by our results. The onset times of response are dependent upon the applied concentration (12), an observation in agreement visually-assessed erythema onset times as reported elsewhere (6, 15, 27–30). It appears likely, therefore, that for methyl nicotinate the response delay (t_L) reflects the time necessary for sufficient drug to penetrate the stratum corneum at a level necessary to produce observable vasodilatation.

The results in Table II indicate how manipulation of the area and time of methyl nicotinate application may be useful to further probe the dose-response and local kinetic behavior of the penetrant. Furthermore, while there is a good agreement between PPG and LDV results in this study, the two techniques generate different types of information about cutaneous microvasculature perfusion, and this differentiation should be exploited.

In summary, it appears (a) that LDV and PPG can successfully monitor the time-course of a local pharmacologic effect elicited by a transdermally delivered vasodilative drug, and (b) that the pharmacodynamic profile may be analyzed to provide some quantitative assessment of the associated kinetics of percutaneous absorption.

Acknowledgements

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