Arch Dermatol Res (1989) 281:95–98

Archives of Dermatological Research

Cutaneous responses to topical methyl nicotinate in black, oriental, and caucasian subjects

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Summary. The response of human skin to topical methyl nicotinate (MN) has been monitored in black, oriental, and caucasian subjects. The study aimed to address the question: "Do racial differences in percutaneous absorption and microcirculatory sensitivity exist?" MN-induced vasodilatation was assessed visually and by laser Doppler velocimetry (LDV). At three dose levels, in the three subject populations, four parameters were compared: (a) the diameter of the maximum visually perceptible erythematous area (E_{mx}); (b) the area under the erythematous diameter versus time curve (AUE); (c) the maximum LDV response (L_{mx}); and (d) the area under the LDV response versus time curve (AUL). At $p < 0.05$, AUL (black) > AUL (caucasian) for all MN concentrations; AUL (oriental) > AUL (caucasian) for the higher dose levels. E_{mx}, AUE and L_{mx} showed no significant differences between races within concentrations. For all subjects, E_{mx}, AUE, and AUL were significantly dependent on MN dose whereas L_{mx} was not. The results suggest that some racial differences in response to topical MN exist and that perception of these distinctions may depend upon the method of measurement.

Key words: Methyl nicotinate – Percutaneous absorption rates – Microcirculatory sensitivity

The objective of the work reported here was to assess the cutaneous response to topically applied methyl nicotinate in black, caucasian, and oriental subjects. The vasodilatory effect of the drug was followed visually, in the conventional manner, by observing erythema, and instrumentally using laser Doppler velocimetry, an optical technique sensitive to changes in skin blood flow. The approaches were designed to probe both the kinetics and the duration of the pharmacological response in different skin types and to elucidate, therefore, comparative information about both percutaneous transport [2, 7, 9, 10–12] and microvasculature sensitivity [3, 13, 14, 17].

Methods

Human subjects

Participants in the study were recruited from the University of California, San Francisco campus community. The subjects were normal, healthy adults, aged 20–35 years; they were non-smokers taking no prescription medication and with no history of skin diseases. The subjects were required to read and sign, prior to entering the study, a human experimentation consent form approved by the UCSF Committee on Human Research. There were five subjects in each of the three racial groups: black, caucasian, and oriental. The black subjects were American negroes of skin type V or VI [8]. The oriental group comprised individuals of Chinese extraction only (skin type IV). The caucasians were white Americans of European background with skin type II. The purity of ethnicity of the groups was such that all four grandparents of each subject demonstrated identical racial characteristics.

Treatment

The vasodilatory response of human skin to methyl nicotinate was followed. The chemical was administered to the upper third of the ventral forearm in aqueous solution. Three concentrations were studied: 1.0 *M*, 0.3 *M*, and 0.1 *M* (approximately 13.7%, 4.6%, and 1.4% w/v, respectively). Solutions were applied using absorbent filter paper discs (1 cm diameter), which prevented liquid from running and spreading over the skin surface. Contact between skin and solution was maintained for 15 s; at the end of this period, the saturated patch was removed and excess solution on the skin was wiped away with tissue.

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Table 1. Comparison of pharmacodynamic response criteria (mean ± SEM) in all subjects (n = 15, three replicates each) between different methyl nicotinate concentrations

Response criterion	Methyl nicotinate concentration (M)		
	0.1	0.3	1.0
E_{mx} (cm)	2.25 ± 0.15	3.07 ± 0.16[a]	3.60 ± 0.18[b]
AUE (cm · h)	2.20 ± 0.17	3.22 ± 0.18[a]	3.68 ± 0.20[b]
L_{mx} (mV)	312 ± 24	348 ± 27	348 ± 27
AUL (mV · h)	205 ± 29	235 ± 30[c]	258 ± 34[d]

[a] 0.3 M response is significantly greater than the 0.1 M response at the $p < 0.01$ level
[b] 1.0 M response is significantly greater than both the 0.3 M and 0.1 M response at the $p < 0.01$ level
[c] 0.3 M response is significantly greater than the 0.1 M response at the $p < 0.05$ level
[d] 1.0 M response is significantly greater than the 0.1 M response at the $p < 0.01$ level

Pharmacodynamic assessment

Vasodilatation was quantified visually and by laser Doppler velocimetry (LDV). Subjective measurements involved periodic evaluation (by a single observer) of the reddened skin area and the determination of a mean erythematous diameter. LDV data were collected with a commercially produced instrument (Medpacific LD 5000 Capillary Perfusion Monitor, Medpacific, Seattle, Washington), which has been described in detail [6]. The perfusion monitor was zeroed on each individual subject according to the manufacturer's specifications.

LDV measurements obtained post-methyl nicotinate application were corrected by subtraction of the basal perfusion value obtained from the forearm skin site prior to the beginning of the experiment. A basal perfusion value was defined as the LDV output observed after the subject had rested in the experimental testing area for at least 15 min. Flow by this time was constant to within ± 5 mV. On each subject, LDV and erythema assessments were performed simultaneously using identical contralateral positions. Following removal of the vasodilatory stimulus, LDV recordings were made continuously for 90 min. Erythema measurements were acquired every 5 min over the same period. Readings were taken more frequently during the 15 min following the onset of erythema. Erythema assessments involved physical measurement of the dimensions of the clearly defined red area of skin. Several measurements through the center of the vasodilated region were made and were averaged to give the mean erythematous diameter.

LDV and erythema measurements, for each methyl nicotinate concentration, were made on three separate occasions for each subject in each of the three experimental groups. A period of at least 4 days elapsed between vasodilative tests. Measurements were made in a single well-ventilated room at reasonably constant temperature (23° ± 1.5° C) and relative humidity (50% – 70%).

Results

Four criteria were used to evaluate the vasodilative response of the human subjects to the methyl nicotinate stimulus:

1. The diameter of the maximum visually perceptible erythematous area (E_{mx})
2. The area under the erythematous diameter versus time curve (AUE)
3. The maximum LDV response (L_{mx})
4. The area under the LDV response versus time curve (AUL)

AUE and AUL were calculated by integration of the erythematous diameter versus time and LDV output versus time profiles, respectively.

In Table 1, the values of these four parameters are compared using the Newman Keuls' multiple comparison test [6] for all subjects between the different methyl nicotinate concentrations used. The three replicates on each subject were first averaged and the mean value was used to compute the data presented.

In Tables 2 and 3, E_{mx} and AUE, and L_{mx} and AUL, respectively, are compared (again with the Newman Keuls' test) for each methyl nicotinate concentration between the different subject groups. Again, the mean values of the triplicate tests on each individual at each concentration were used to determine the data shown.

Both erythema diameter and LDV output as a function of time post-application of nicotinate showed similar profiles to those illustrated in earlier publications [1, 4, 5, 8]. The general shape of these profiles was not dependent upon subject group.

Basal LDV-assessed perfusion values fell typically in the range of 20 – 60 mV for all subjects. For any one subject, basal flow varied by less than ± 20 mV. There was no correlation between these values and skin pigmentation [basal flows: black (n = 45) 26.2 ± 20.4 mV; oriental (n = 45) 31.7 ± 12.7 mV; caucasian (n = 45) 35.8 ± 12.3 mV] nor was there a significant relationship between basal flow L_b and maximal flow L_{mx} post-drug application: black (n = 45), $L_{mx} = 295 + 3.3\ L_b$, $r^2 = 0.17$; oriental (n = 45), $L_{mx} = 329 + 1.16\ L_b$, $r^2 = 0.03$; caucasian (n = 45), $L_{mx} = 246 + 0.5\ L_b$, $r^2 = 0.003$.

Discussion

The results in Table 1 consider the dose-response behavior to methyl nicotinate in the entire subject population of mixed racial background. The data are comparable to previously published observations [5, 8]. The maximum magnitude of erythematous area increased significantly with increasing nicotinate concentration and the integrated erythema diameter versus time curve showed the same trend. The spread of erythema is a function of at least three forces: supply of nicotinate from the epidermis, radial transport in the dermis, and irreversible uptake by the microvasculature. The sequence of events probably also includes release of vasoactive prostaglandins [15]. The E_{mx} and AUE values determined in this study show

Table 2. E_{mx} (cm) and AUE (cm · h) values (mean ± SEM) at each methyl nicotinate concentration for three different subject groups

Group (n = 5)	Methyl nicotinate concentration (M)					
	0.1[a]		0.3[a]		1.0[a]	
	E_{mx}	AUE	E_{mx}	AUE	E_{mx}	AUE
Black	2.19±0.50	2.03±0.33	2.99±0.25	3.05±0.35	3.33±0.16	3.35±0.35
Caucasian	2.18±0.22	2.12±0.27	3.25±0.30	3.35±0.33	4.16±0.25	4.03±0.30
Oriental	2.37±0.36	2.47±0.25	2.98±0.34	3.22±0.30	3.31±0.38	3.68±0.37

[a] At this concentration, there are no significant differences (at $p < 0.05$) between the responses (E_{mx} or AUE) of the three different subject groups

Table 3. L_{mx} (mV)[a] and AUL (mV · h) values (mean ± SEM) at each methyl nicotinate concentration for three different subject groups

Group (n = 5)	Methyl nicotinate concentration (M)					
	0.1[a]		0.3[a]		1.0[a]	
	L_{mx}[b]	AUL[c]	L_{mx}[b]	AUL[d]	L_{mx}[b]	AUL[d]
Black	364±50	287±63	380±61	309±63	400±59	331±73
Caucasian	252±22	118±24	276±43	131±23	260±23	139±27
Oriental	324±58	209±30	384±14	265±27	392±16	304±14

[a] L_{mx} values are absolute values minus the basal perfusion levels. The latter were typically in the range 30–80 mV
[b] At this concentration, there is no significant difference (at $p < 0.05$) between the responses of the three different groups
[c] At this concentration, the response of the black group is significantly higher (at $p < 0.05$) than that of the caucasian group
[d] At this concentration, the responses of the black and oriental groups are significantly higher (at $p < 0.05$) than that of the caucasian group

the same trend (and are of similar magnitude) as those in the literature [1, 4]. Over the concentration range studied, L_{mx} was essentially constant, an observation in agreement with a previous study [5], which considered the LDV dose-response behavior (in a caucasian population) to the same methyl nicotinate stimulus over a concentration range of 5–100 mM. Hence, it is clearly demonstrated that the change in skin blood flow, which can be elicited by the topical challenge, is saturable and that the concentrations employed in this work correspond to the plateau region of the dose-response curve. The area under the LDV response-time profile (AUL) increased with increasing applied methyl nicotinate concentration. Despite the constancy of L_{mx}, the duration of local perturbation was prolonged as the thermodynamic activity of drug on the skin surface was raised. Again, these observations are consistent with previous results [5].

In Tables 2 and 3, respectively, E_{mx} and AUE, and L_{mx} and AUL values are compared, for each nicotinate concentration, between the three subject groups. It can be seen that E_{mx}, AUE, and L_{mx} in the different racial populations are similar and are not statistically distinguishable. It is interesting to note that although E_{mx} and AUE appear most sensitive (i.e., show the greatest range of values) to methyl nicotinate in caucasian skin, the mean L_{mx} at each concentration is lowest for this cohort.

Differential responses between the racial groups are revealed for AUL (Table 2). Perhaps somewhat surprisingly, the integrated LDV response versus time in black skin is greater than that in the skin of caucasians. Also, at the higher concentrations (0.3, 1.0 M), AUL (oriental) is significantly higher than AUL (caucasian). Thus, it seems that, although the visual assessment or erythema cannot distinguish between different skin types, the objective LDV procedure does reveal differential responsiveness to the methyl nicotinate stimulus.

Oriental skin, in this study, did not show any dramatic differences in response (other than that indicated in Table 3) from either black or caucasian skin. Clearly, on the basis of the results reported here, a case cannot be made for differential permeabilities of different skin types unless one argues, for example, that poor absorption in one racial group is exactly compensated by increased microvasculature sensitivity. Such a coincidence seems, to us, rather implausible although it must be stated that only a single chemical at three concentrations has been investigated and

that the subject populations were not large. Future experiments, therefore, should consider a concentration range spanning the complete dose-response curve and must involve an increased number of subjects in the different cohorts tested.

Acknowledgements. We thank Dr. M. Tur for assistance with data analysis and Mrs A. Mazel for manuscript preparation. Financial support was provided by Ciba-Geigy and by N.I.H. grant GM-33395 to RHG, who was the recipient of a Special Emphasis Research Career Award (1-KO1-OH-00017) from the National Institute of Occupational Safety and Health.

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Received January 11, 1988