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# CUTANEOUS RESPONSES TO TOPICAL METHYL NICOTINATE IN BLACK, ORIENTAL AND CAUCASIAN SUBJECTS

Constantine J. Gean, M.D.[1], Ethel Tur, M.D.[2], Howard I. Maibach, M.D. and Richard H. Guy, Ph.D.[3]

Departments of Pharmacy, Pharmaceutical Chemistry and Dermatology
Schools of Pharmacy and Medicine
University of California, San Francisco
San Francisco, CA. 94143.

[1]Present address: School of Medicine, University of Maryland, Baltimore, MD.

[2]Permanent address: Department of Dermatology, Ichilov Hospital, Tel Aviv, Israel.

[3]To whom correspondence should be addressed.
(926-S), University of California, San Francisco, CA 94143.
(415)-476-4830

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16. Abstract (Limit: 200 words)

Assessments were made of cutaneous responses to topically applied methyl-nicotinate (93607) (MN) on black, oriental, and Caucasian subjects to determine if racial differences had any part to play in percutaneous absorption and microcirculatory sensitivity. Both visual and laser Doppler velocimetry evaluations were made of the MN induced vasodilatation. Specific assessment was made of the diameter of the maximum visually perceptible erythematous area, the area under the erythematous diameter versus time curve, the maximum laser Doppler velocimetry response, and the area under the laser Doppler velocimetry response versus time curve. For all subjects each of the measures above-mentioned was dependent on the dose of MN applied with the exception of the maximum laser Doppler velocimetry response. The authors conclude that although some racial differences did appear to exist in response to topical MN, the perception of these distinctions may depend on the method of measurement.

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**Synopsis**

Cutaneous responses to topical methyl nicotinate were assessed visually and by laser Doppler velocimetry in black, oriental and caucasian subjects. Racial differences in microvascular response were observed but the perception of these distinctions may depend upon the method of measurement.

**Abstract**

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.

## INTRODUCTION

The skin represents the most important interface between man and his surrounding environment. Evolution has resulted in a diverse population of human racial types, which are most superficially recognized by their different skin pigmentation. These differences are crucial to the practising dermatologist who must be able to assess accurately disease severity in patients of a broad ethnic background and who must, therefore, develop a sense of how a particular symptom will be manifested in, for example, caucasian versus black skin. The same variability in skin types and color are also of clear importance to the investigative cutaneous scientist, who, in conducting experiments designed to answer questions about skin, frequently requires a human subject population in which to test hypotheses. The investigator must decide whether the experimental cohort is most appropriately limited to a single skin type or should be broadened (and, hence, increased in number) to include a range of subjects from various racial origins.

How much then do we know about skin differences between individuals from different ethnic backgrounds? On the whole, the answer to this question is 'not very much'. Histologically, it is apparent that human skin is composed of the same basic structures regardless of race. Clearly, pigmentation is a variable and, as a result, skin reflectance, for example, is a function of skin color.[1-3] It has, furtheremore, been reported that the integrity of the stratum corneum of blacks is greater than that of caucasians[4,5] and it has been suggested that this observation is the result of the greater apparent microscopic compactness of the black skin horny layer. This conclusion seemed

consistent with a perception that black skin resists topical irritants better than caucasian skin[6] and led to the belief that the diffusional barrier of black stratum corneum is greater than the corresponding white resistance. This hypothesis has been tested *in vivo*[7,8] and *in vitro*[9] and has been both substantiated and refuted.

A second area of investigation has centered upon the magnitude of skin flushing reactions in Mongoloids versus caucasians.[10,12] Increased alcohol-induced flushing has been established in Japanese, Chinese and North American Indian subjects over that seen in caucasians and this sensitivity has been intensely studied and recently reviewed.[13] In other respects, however, (for example, barrier function and sensitivity to topical irritation) oriental skin has not been well studied and little comparative information with caucasian and black skin has been derived.

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 and microvasculature sensitivity.

## 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 disease. 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 5 subjects in each of the three racial groups: black, caucasian and oriental. The black subjects were American negroes of skin type V or VI[14]. The oriental group comprised individuals of Chinese extraction only (skin type IV). The caucasians were white Americans of European background with skin type II.

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 concentration were studied: 1.0M, 0.3M and 0.1M (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 seconds; at the end of this period, the saturated patch was removed and excess solution on the skin was wiped away with tissue.

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 Corp., Seattle, WA), which has been described in detail.[15] The perfusion monitor was zeroed on each individual subject according to the manufacturer's specifications. This procedure is supposed to equalize the instrument's sensitivity on skins of different pigmentation and reflectance. While this objective is difficult to quantify absolutely, the operation does ensure that, pre-experiment, the baseline measurement for all subjects is obtained from the same amount of information received by the efferent optical fiber of the LDV.

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 minutes. 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 minutes. Erythema measurements were acquired on average every five minutes over the same period. Readings were taken more frequently during the 15 minutes following the onset of erythma. 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 four 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 I, the values of these four parameters are compared using the Newman Keuls' multiple comparison test,[16] 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 II-V, $E_{mx}$, AUE, $L_{mx}$ 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.[17-20] 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), $L_{mx} = 246 + 0.5\ L_b$, $r^2 = 0.003$.

COMMENT

It should first be noted that a 15 second exposure of human skin to aqueous solutions of methyl nicotinate, in the concentration range studied, elicits significant pharmacological effect and implies significant percutaneous absorption. Previous work has shown that nicotinic acid esters penetrate skin efficiently from aqueous solution but that the parent acid does not[21]. The latter observation reflects the poor partitioning of the highly water soluble acid into the lipophilic stratum corneum. Detailed analysis of erythema onset times, as a function of concentration and application time following topical nicotinate administration, has demonstrated that these molecules probably transport across the stratum corneum via the lipid-filled intercellular channels[22,23].

The results in Table I consider the dose-response behavior to methyl nicotinate in the entire subject population of mixed racial background. The data are, we believe, consistent with expectations and, in general, comparable to previously published observations. The maximum magnitude of erythematous area increased significantly with increasing nicotinate concentration and the integrated erythema diameter versus time curve showed the same trend. It has been shown that the radial spread of erythema, induced by topical methyl nicotinate application, is facilitated via a dermal capillary transport and re-equilibration process.[17,18] The rapidity of movement of the erythematous reaction is too great to be accounted for by simple drug diffusion in the dermis. The spread of erythema is thus a function of three forces: supply of nicotinate from the epidermis, radial transport

in the dermis, and irreversible uptake by the microvasculature. The $E_{mx}$ and AUE values determined in this study show the same trend (and are of similar magnitude) as those in the literature[17,18] and lend support, therefore, to the proposed transport mechanism. Over the concentration range studied, $L_{mx}$ was essentially constant, an observation in agreement with a previous study from our laboratory[19] 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 our previous results.[19]

In Tables II, III and IV, respectively, $E_{mx}$, AUE and $L_{mx}$ values are compared, for each nicotinate concentration, between the three subject groups. It can be seen that these responses 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.

In Table V, differential responses between the racial groups are

revealed for AUL. 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 of erythema cannot distinguish between different skin types, the objective LDV procedure does reveal differential responsiveness to the methyl nicotinate stimulus.

Preliminary experiments in our laboratory with LDV[24] had revealed no difference in AUL between caucasian and black subjects following a 15 second exposure to 0.1 M methyl nicotinate. However, at that time, the zero surface calibration procedure* was not available for the LDV and this may have resulted in an under-estimation of the AUL in the earlier black subject group. It should also be noted that $L_{mx}$ and AUL values in the black cohort are associated with the largest intersubject variability. One possibility may be differences in the degree of pigmentation; however, within our limited experience, we have detected no correlation between depth of black skin color and extent of pharmacodynamic response. It may be suggested, perhaps, that greater inherent inter-individual differences are to be found in black skin and that this may account for the disparate observations in the literature concerning the barrier to molecular absorption across black skin.[4,7-9] Importantly, both the visual measurements of erythema and the objective assessments with LDV of the black skin response are not supportive of

*Medpacific Corp., Seattle, WA., technical information.

the view that 'black skin resists chemical irritation better than less pigmented skin.' Data substantiating this conventional hypothesis are based only on visual determinations.[5,6] It has been found here that careful subjective and objective measurements are in agreement and that the only major significant difference between black skin and white skin indicates a greater responsiveness in the former. It should be pointed out that erythematous area rather than intensity was chosen in this work as the principal subjective indicator. It is the experience of this laboratory that visual determination of response versus no response is more reliable than attempts to evaluate gradations of response (e.g. erythema ratings as +1 versus +2, etc.).

Oriental skin, in this study did not show any dramatic differences in response (other than that indicated in Table V) 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 (a conclusion which will be tested in further studies which will consider intradermal delivery of the chemical stimulus). Of course, it must be stated that only a single chemical at three concentrations has been investigated and that the subject populations are not large. Future work must address a range of chemicals of different physicochemical properties, delivered from various vehicle preparations at concentrations covering the complete dose-response curve and must involve an increased number of subjects in the different cohorts tested.

Table I. Comparison of pharmacodynamic response criteria (mean ± SE) 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.hr) | 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.Hr) | 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]1.0M response is significantly greater than both the 0.3 M and 0.1 M responses 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.

Table II. $E_{max}$ (cm) values (mean ± SE) 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] |
| Black | 2.19 ± 0.5 | 2.99 ± 0.25 | 3.33 ± 0.16 |
| Caucasian | 2.18 ± 0.22 | 3.25 ± 0.30 | 4.16 ± 0.25 |
| Oriental | 2.37 ± 0.36 | 2.98 ± 0.34 | 3.31 ± 0.38 |

[a]At this concentration, there is no significant difference (at $p < 0.05$) between the responses of the three different groups.

Table III. AUE (cm.hr) values (mean ± SE) 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] |
|---|---|---|---|
| Black | 2.03 ± 0.33 | 3.05 ± 0.35 | 3.35 ± 0.35 |
| Caucasian | 2.12 ± 0.27 | 3.35 ± 0.33 | 4.03 ± 0.30 |
| Oriental | 2.47 ± 0.25 | 3.22 ± 0.30 | 3.68 ± 0.37 |

[a]At this concentration, there is no significant difference (at $p < 0.05$) between the responses of the three different groups.

**Table IV.** $L_{mx}$ (mV) values (mean ± SE)[a] at each methyl nicotinate concentration for three different subject groups.

| Group (n=5) | Methyl Nicotinate Concentration (M) 0.1[b] | 0.3[b] | 1.0[b] |
|---|---|---|---|
| Black | 364 ± 50 | 380 ± 61 | 400 ± 59 |
| Caucasian | 252 ± 22 | 276 ± 43 | 260 ± 23 |
| Oriental | 324 ± 38 | 384 ± 14 | 392 ± 16 |

[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.

Table V. AUL (mV.hr) values (mean ± SE) at each methyl nicotinate concentration for three different subject groups.

| Group (n=5) | Methyl Nicotinate Concentration (M) 0.1[a] | 0.3[b] | 1.0[b] |
|---|---|---|---|
| Black | 287 ± 63 | 309 ± 63 | 331 ± 73 |
| Caucasian | 118 ± 24 | 131 ± 23 | 139 ± 27 |
| Oriental | 209 ± 30 | 265 ± 27 | 304 ± 14 |

[a]At this concentration, the response of the black group is significantly higher (at $p < 0.05$) than that of the caucasian group.

[b]At this concentration, the responses of the black and oriental groups are significantly higher (at $p < 0.05$) than that of the caucasian group.

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