

Racial Differences in Pharmacodynamic Response to Nicotinates *In vivo* in Human Skin: Black and White

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This study evaluated the vasodilation induced in young whites and blacks by topical application of two nicotinates, methylnicotinate (MN) and hexylnicotinate (HN) at the same concentration and in the same vehicle. To assess the influence of skin surface lipids and water content of the stratum corneum on the penetration of the substances, the drugs were applied on the back, on untreated skin and pre-occluded and pre-delipidized sites. Skin blood flow was monitored with laser Doppler velocimetry. The initial response recorded at 15 min (IR), the peak response (PR) and the area under the curve (AUC) were used to characterize the pharmacodynamic response. Statistically significant racial differences in the penetration of nicotinates were detected for the area under the response curve in the untreated and occluded sites, for the initial response and peak response in the pre-occluded site. Occlusion increased (even though not significantly) penetration, except for blacks in the methylnicotinate experiment. Delipidization elicited significantly lower responses for the IR and PR in the MN study, rendering the penetration similar in the two groups. No major differences were recorded between the two nicotinates. The effect of delipidization was most noticeable in blacks in the MN study. We suggest that there are racial (blacks vs. whites) differences in percutaneous penetration of nicotinates, with decreased levels in black skin recorded in all sites investigated. **Key words:** Blood flow; Laser Doppler velocimetry.

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Guy et al. (1) reported a decreased maximum response in young blacks vs. whites recorded with photoplethysmography after topical application of 100 mM methylnicotinate in aqueous solution. No major differences were detected when laser Doppler velocimetry was used and the authors concluded that there is a similar response to MN in the different skin types.

Our study evaluated the induced vasodilatation in young whites and blacks after topical application of two nicotinates with different physical chemical properties, namely methylnicotinate (MN) and hexylnicotinate (HN) at the same concentration and in the

same vehicle. To assess the influence of skin surface lipids and water content of the stratum corneum on the penetration of the substances, the drugs were applied on the untreated back skin of the subjects and in sites on the back where two pre-treatments were performed: *a*) occlusion: as recently reported (2), short-term occlusion seems to increase transcutaneous penetration; *b*) delipidization: skin surface lipids may play an important role in penetration (3).

We monitored skin blood flow utilizing laser Doppler velocimetry (LDV) to quantify cutaneous microcirculation (4). LDV measures skin blood flow responses to nicotinates. It can be used to quantify the time course of percutaneous absorption. Our data reveal differences between whites and blacks, reflected by the differing responses to HN and MN, or the response to the pre-treatments.

MATERIALS AND METHODS

Nine white (age 30.6, standard deviation ± 8.8) and 10 black (age 29.9, S.D. ± 7.2) male volunteers entered the study. The subjects rested for 30 min and were asked to avoid strenuous activity, alcohol ingestion and smoking for 12 h prior to the procedures. The test was performed on the back in three areas: *a*) untreated skin, *b*) skin occluded with an impermeable plastic for half an hour (Parafilm, American Can Co. Greenwich, Conn., USA). The nicotinates were then applied and water content (WC) was measured by a capacitance device (Facial aqua-meter, IBS Co. Ltd, Japan). The technique measured the stratum corneum water content in a few seconds by applying a 1 cm diameter probe on the skin surface (5). *c*) delipidized skin: skin surface lipids were solubilized by applying ethyl acetate (Baker analysed, Phillipsburg, NJ) for 3 min via a cotton swab without scrubbing the skin surface. To assess satisfactory delipidization, casual sebum levels were determined prior and after ethyl acetate treatment, using a Sebumeter (Schwarzaupt, Köln, West Germany) (6).

The nicotinate-induced vasodilatation was assessed by using laser Doppler velocimetry (Medpacific LD 5000 Capillary Perfusion Monitor, Medpacific Inc., Seattle, WA). The pharmacodynamic response to a 5-min application of 10 mM methylnicotinate and 10 mM hexylnicotinate in 60:40 water isopropylalcohol vehicle was measured. Administration of nicotinates was achieved via a filter paper disk saturated with the solution. After the 5-min application of the drug, LDV readings were taken every 10 min for 1 hour. The initial response recorded at 15 min (IR), the peak response (PR), and the area under the curve (AUC) allowed characterization of the pharmacodynamic response. Statistical analysis of the data used ANOVA and Fisher's test.

Table I. Racial differences in transcutaneous penetration of nicotines

Values are expressed in millivolts (mean \pm standard deviation). Statistically significant differences are detectable between the two races in the area under the curve response (untreated and pre-occluded sites), in the initial response and in the peak response (occluded sites)

Racial differences in pharmacodynamic response to methyl and hexyl nicotine

Area under the curve		methyl	hexyl
Untreated	White	1 039 \pm 220	1 112 \pm 642
	Black	689 \pm 299	886 \pm 404
			<i>p</i> =0.04
Occluded	White	1 060 \pm 215	1 015 \pm 242
	Black	647 \pm 331	800 \pm 405
			<i>p</i> =0.004
Delipidized	White	793 \pm 582	760 \pm 544
	Black	565 \pm 175	788 \pm 416
			N.S.
Initial response			
Untreated	White	379 \pm 111	285 \pm 178
	Black	313 \pm 164	238 \pm 118
			N.S.
Occluded	White	411 \pm 83	354 \pm 107
	Black	288 \pm 150	268 \pm 134
			<i>p</i> =0.01
Delipidized	White	317 \pm 219	257 \pm 128
	Black	210 \pm 89	214 \pm 101
			N.S.
Peak response			
Untreated	White	440 \pm 98	378 \pm 199
	Black	334 \pm 136	311 \pm 96
			N.S.
Occluded	White	471 \pm 93	397 \pm 74
	Black	298 \pm 144	315 \pm 147
			<i>p</i> =0.006
Delipidized	White	336 \pm 217	293 \pm 140
	Black	246 \pm 76	274 \pm 96
			N.S.

RESULTS

The results are shown in Tables I and II. Casual sebum level was 121 μ g (\pm 79) in whites and 72 μ g (\pm 31) in blacks. The difference was not statistically significant. Delipidization produced significant lowering of casual levels (38.5 \pm 5.7 in whites, 43.2 \pm 12 in blacks) (*p*<0.01 in whites and blacks). Occlusion increased the water content of the stratum corneum from 5.9 \pm 2.8 to 25.1 \pm 21.3 in whites (*p*<0.01) and from 7 \pm 3.4 to 20.4 \pm 16.5 in blacks (*p*<0.02). Lower

Table II. Effect of the pre-treatments on penetration of nicotines

Methyl nicotine responses were sensitive to delipidization

Peak response Methyl nicotine

Whites

Untreated 440 \pm 98

Occluded 471 \pm 93

Delipidized 336 \pm 217

The delipidized site was significantly different from the occluded and untreated (*p*<0.05)

Blacks

Untreated 334 \pm 136

Occluded 298 \pm 144

Delipidized 246 \pm 76

The delipidized site was significantly different from the untreated (*p*<0.05)

Initial response

Methyl nicotine

Blacks

Untreated 313 \pm 164

Occluded 288 \pm 150

Delipidized 210 \pm 89

The delipidized site was significant different from the untreated and occluded (*p*<0.05)

LDV levels were recorded in blacks throughout the study. No significant differences were recorded between the two nicotines.

Occlusion increased the levels, except for MN in blacks. Significant racial differences were recorded for the area under the curve response in the untreated and pre-occluded sites (*p*=0.04 and *p*=0.004, respectively); for the initial response and peak response in the pre-occluded site (*p*=0.01 and *p*=0.006, respectively). The effects of the pre-treatment were detectable within each group in the MN study, where delipidized sites were different from untreated skin for the initial response in blacks and the peak response in both races at the *p*<0.05 level.

DISCUSSION

In terms of percutaneous absorption, different results between black and white skin have been obtained in vivo and in vitro, probably depending on the technique.

In vivo, skin penetration of diflorasone acetate (7) was the same in black and white subjects, while dipyrithione showed 34% less penetration in blacks. In vitro, the absorption of fluocinolone acetonide was higher in white skin (8).

Differences in the ability of black and white skin to react to irritants have been attributed mainly to permeability. Other factors, such as metabolism, mediators of inflammation, ability of the microcirculation to eliminate the drug, and blood vessel reactivity could be involved. The conventional thought that black skin resists chemical irritation better than white (9) has been substantiated by studies on the anatomic-physical properties of black and white skin. Blacks require a significantly greater number of cellophane tape strippings than whites, to remove the stratum corneum and the number of corneum layers is greater. The average of the stratum corneum thickness is the same in two races, indicating that the black stratum corneum is more compact. Sucrose density gradient centrifugation determination revealed greater density for black stratum corneum (10). When skin lipid effects were minimized by organic solvent mixtures, the converse was noted. Other authors reported a higher lipid content in black stratum corneum (11), which would not explain the diminishing density for the latter observed in organic solvents by Weigand & Gaylor (9).

Our data suggest a decreased susceptibility of black skin to nicotines and confirm the previous report of Guy et al. (1). The comparison between blacks and whites revealed lower values in the black group throughout the study. ANOVA shows a significant decrease in LDV levels for the area under the curve response in the untreated ($p=0.04$) and pre-occluded sites ($p=0.004$); for the initial response in the occluded site ($p=0.01$); for the peak response in the pre-occluded site ($p=0.006$). No significant differences were recorded in the responses to the two nicotines. These data substantiate a reduced penetration and/or a reduced reactivity of blood vessels to nicotines in the black group. In a previous study, utilizing the same experimental model, we found no difference between white and hispanic skin (12).

The pre-treatment with delipidization reduced all parameters considered in the two groups. The LDV-assessed nicotine response was similar in the two races. The results recorded after delipidization were characterized by high standard deviation levels for the AUC values, rendering the response in these sites significantly different only in the methyl nicotine study for the peak response in whites and blacks, and for the initial response in blacks. It is possible that skin surface lipids play a role in modulating initial penetration of nicotines into the skin, but affect to a lesser extent the whole amount of chemical that pene-

trates the skin. The effect of delipidization was most noticeable in blacks in the MN study. These data are difficult to interpret; HN is a lipophilic compound and even small quantities of lipids on the skin surface allow penetration. Statistically significant differences between the two races were recorded (with more relevant data in the white group) when penetration was enhanced with occlusion (all parameters, see Tables). Lastly, in blacks, in the MN experiment, a significantly lower response ($p<0.05$) was recorded at the delipidized site as measured by the peak response and the initial response (Table II).

The data presented are in general agreement with the conventional belief that black skin resists the penetration of chemicals better than white. Differential effects on penetration due to occlusion (hydration of the stratum corneum) and delipidization (barrier modulation) are suggested by the results. While neither black nor white skin discriminated the different physicochemical properties of the test compound used, it is clearly premature to draw general conclusions from this initial finding. Obviously, this relatively uninvestigated area of physiology and pharmacology requires considerable work. The simple and non-invasive technique of LDV offers a potentially effective way both to evaluate percutaneous absorption in black and white skin of a diverse way of chemicals, and to increase our understanding of skin physiology with respect to optimization of drug delivery to discrete patient populations.

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REFERENCES

1. Guy RH, Tur E, Bierke S, Maibach HI. Are there age and racial differences to methyl nicotine-induced vasodilatation in human skin? *J Am Acad Dermatol* 1985; 12: 1001-1004.
2. Ryatt KS, Stevenson JM, Maibach HI, Guy RH. Pharmacodynamic measurement of percutaneous penetration enhancement in vivo. *J Pharm Sci* 1986; 75: 374-377.
3. Menczel E. Skin delipidization and percutaneous absorption. In: Bronaugh RL, Maibach HI, eds. *Percutaneous Absorption*. New York: Marcel Dekker Inc, 1985: 133-139.
4. Stevenson JM, Maibach HI, Guy RH. Laser Doppler photoplethysmographic assessment of cutaneous micro-

- vasculature. In: Maibach HI, Lowe NJ, eds. *Models in Dermatology* 1987. Basel: Karger, 1987: 121.
5. Tagami H, Ohi M, Iwatsuki K, Kanamaru Y, Yamada M, Ichijo B. Evaluation of skin surface hydration in vivo by electrical measurement. *J Invest Dermatol* 1980; 75: 500–507.
 6. Dikstein S, Harzshark A, Bercovici R, Orgad A. Distribution of sebum measurement in normal adult woman. Abstract, IV Int. Symp. on Bioengineering and the skin, Besancon, France, Sept. 1983.
 7. Wickema-Sinha WJ, Shaw SR, Weber OJ. Percutaneous absorption and excretion of tritium-labelled diflorasone diacetate, a new topical corticosteroid, in the rat, monkey and man. *J Invest Dermatol* 1978; 7: 372–377.
 8. Stoughton RB. In: Montagna W, Stoughton RB, Van Scott EJ, eds. *Pharmacology of the skin*. New York: Appleton-Century-Crofts, 1969: 542.
 9. Weigand DA, Gaylor JR. Irritant reaction in Negro and Caucasian skin. *South Med J* 1974; 67: 548–551.
 10. Weigand DA, Haygood C, Gaylor GR. Cell layers and density of Negro and Caucasian stratum corneum. *J Invest Dermatol* 1974; 62: 563–568.
 11. Rienertson RP, Wheatley VR. Studies on the chemical composition of human epidermal lipids. *J Invest Dermatol* 1959; 32: 49–51.
 12. Berardesca E, Maibach HI. Effect of race on percutaneous penetration of nicotines in human skin: a comparison of white and hispano-americans. *Bioeng Skin* 1988; 4: 31–38.

Studies on the Time Course of Dithranol-induced Inflammation by Quantification of Alkaline Phosphatase

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An inflammatory response of the skin to dithranol-induced free radicals seems to be essential for its clinical efficacy. In normal volunteers this response was evaluated at the level of the microvasculature following 30 min, 2 h and 24 h applications, using a functional parameter (erythema) and a biochemical parameter (alkaline phosphatase). The results of 'short contact' and 24 h applications were similar. In all schedules a maximum erythema was seen 2–3 days after the application which had resolved totally after 6–8 days. A marked discrepancy was established between the duration of functional and biochemical abnormalities; the alkaline phosphatase activity reached a maximum 1 day after the culmination of the erythema and persisted up to at least 7 days after disappearance of the erythema. These findings are discussed in the light of the day-to-day management of psoriasis with dithranol. Key words: Anthralin; Endothelium; Psoriasis; Enzymology.

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Dithranol is a well-established treatment for chronic plaque psoriasis (1, 2). Although its antipsoriatic working mechanism has not yet been clarified, the induction of free radicals is supposed to play a central role (3). In the management of psoriasis an adequate

concentration adjustment is a *conditio sine qua non* for therapeutic success. Concentrations too low lack clinical efficacy, concentrations too high induce unpleasant irritation of the skin. Therefore, the irritative potential of dithranol is a crucial issue.

Studies on dithranol-induced irritancy have been carried out using several approaches: clinical assessment of erythema (4, 5), reflectance photometry (6), measurement of skin contact temperature by thermometry or by thermography (5, 7, 8), measurement of superficial blood flow by laser-Doppler flowmetry (5) and measurement of oedema by Harpender calipers (9). A direct assessment of inflammation of the skin is possible by fluorometric quantification of alkaline phosphatase (ALP) in biopsies (10, 11). ALP is a marker enzyme for the ascending capillary loops (12). In experimental inflammation of the skin and inflammatory dermatoses the expression of ALP in the endothelial cells is increased substantially above values observed in non-inflamed skin and infiltrate cells of different origin show a mild expression of the enzyme (13).

The aim of the present investigation is to investigate the time course of dithranol-induced irritation of the skin at the level of the microvasculature by visual assessment of erythema and by measuring the activity of the marker enzyme ALP after 24 h applications, 2 h applications and 30 min applications of the drug on normal skin.