Effect of Cutaneous Hypoxia upon Erythema and Pigment Responses to UVA, UVB, and PUVA (8-MOP + UVA) in Human Skin*

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The effect of oxygen deprivation upon UVA-, UVB-, and PUVA-induced pigment and erythema responses in normal human skin was examined. Before exposure, varying degrees of hypoxia in the skin of the forearm were achieved by inflating a sphygmomanometer cuff applied to the upper arm. After the transcutaneously measured pO_2 had stabilized, sites on the inner forearm were exposed to UVA, UVB, or 8-MOP + UVA radiation, to determine dose thresholds for the induction of erythema and pigmentation at different cuff pressures. Inflation of the cuff to greater than systolic pressure completely inhibited immediate and delayed pigment responses (IPD, DT) to UVA doses greater than 10 times the normal pigmentation threshold dose. UVA-induced delayed erythema responses were partially inhibited by cuff inflation: 2.7 times the minimal erythema dose of UVA was necessary to cause an erythema response when exposure occurred during vascular occlusion. In contrast, erythema and pigment responses to UVB and PUVA were unaltered by cuff pressures exceeding systolic pressure during exposure. Inhibition of UVA-induced erythema and pigment responses by vascular occlusion were reversed by the transcutaneous diffusion of 100% O_2. These findings indicate that the cutaneous responses to UVA and UVB occur by separate pathways differing with respect to O_2 dependence. Our findings agree with those of other studies which indicate that PUVA-induced phototoxicity and melanogenesis are not O_2-dependent. *J Invest Dermatol 86: 649–652, 1986*

Oxygen, while essential for cellular respiration, can also mediate damage via photodynamic action (photosensitized oxidation). This is a photoreaction which requires the presence of both a sensitizer, either endogenous or exogenous, and oxygen [1]. In several biologic systems, the harmful effects of UVA (320–400 nm) radiation, unlike UVB (290–320 nm) radiation, appear to be mediated by photodynamic processes; bacterial inactivation by UVA is markedly enhanced by oxygen in contrast to UVB-induced photodamage which is O_2-independent [2]. A similar oxygen dependence of UVA lethality has been shown for yeast [3] and 2 mammalian cell lines [4]. UVA-induced corneal damage is likewise enhanced by O_2 [5].

Similar observations have been made in human skin in vivo. Early reports noted that erythema and pigmentation responses elicited by UV radiation shorter than 320 nm were not inhibited when circulation to the forearm was occluded during irradiation [6]. Other studies have demonstrated that the induction of pigment darkening by UVA and visible light is prevented by vascular occlusion during exposure [7–9]. More recently it has been observed that pressing a transparent acrylic plate against the skin during UVA irradiation can inhibit not only pigment darkening but also the delayed erythema and melanogenesis responses, supposedly by occluding cutaneous blood flow [10]. Although these reports did not document a decrease in pO_2 during UV irradiation, they provide qualitative evidence that UVA-induced erythema and pigmentation may obey a photodynamic principle.

We have measured the effect of cutaneous pO_2 alteration upon the induction of erythema and pigment responses to UVA, UVB, and topical 8-methoxypsoralen (MOP) sensitization + UVA (PUVA).

MATERIALS AND METHODS

Subjects Twenty normal volunteer subjects were employed for this study. Informed consent was obtained from each. For the study of pigment responses, 10 individuals of Skin Types 3 and 4 were employed, while for study of erythema responses 10 subjects of Skin Types 1 and 2 were used.

Radiation Sources For UVA exposures, the filtered output of a 2000-W xenon arc with a f/1.5 quartz condensing lens system was employed. The beam was filtered through 6 cm of an aqueous solution of 7% copper sulfate and 7% cobalt sulfate, followed by a 2-mm WG 345 filter, to reduce infrared (IR) and UVB wave-

Manuscript received October 15, 1985; accepted for publication January 10, 1986.

Supported by the Arthur O. and Gullan M. Wellman Foundation.

*This work was presented in part at the Annual Meeting of The Society for Investigative Dermatology, Inc., Washington, D.C., May 7–9, 1984 (J Invest Dermatol 82:420, 1984).

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Abbreviations:
IPD: immediate pigment darkening
IR: infrared
MED: minimum erythema dose
MMD: minimum melanizing dose
8-MOP: 8-methoxypsoralen
MPD: minimum phototoxic dose
PUVA: psoralen plus UVA
tcO_2: transcutaneous oxygen tension
UVA: ultraviolet A (320–400 nm)
UVB: ultraviolet B (290–320 nm)

lengths. The beam was then reflected from an IR-transmitting UV-reflecting dichroic mirror vertically onto the patient. An Eppley thermopile and an International Light (IL 782) spectroradiometer were used to measure the irradiance at the skin surface: an irradiance of approximately 60 mW/cm^2 was employed. Peak emission was at 360 nm. When examining PUVA-induced responses the irradiance was reduced to 6 mW/cm^2. UVB was administered from a bank of 12 FS36 fluorescent sunlamp tubes, providing 0.8 mW/cm^2 at the skin surface. Peak emission was at 315 nm.

Induction of Ischemia A sphygmomanometer cuff was applied to the upper arm of the subject and inflated to 30, 60, 80, 100, 120, or 150 mm Hg. The cuff was inflated for a 5-min period before exposure, to allow the pO_2 to stabilize. For exposures requiring greater than 15 min to complete, the cuff was deflated for a 5-min rest period after each 15 min of exposure. The cuff was then reinflated for a 5-min equilibration period before continuing the exposure. The effects of the cuff inflation were monitored using a transcutaneous oxygen probe.

Transcutaneous pO_2 Monitoring Transcutaneous oxygen tension (tcO_2) was monitored by a Clark type cutaneous sensor (Roche-Kontron) [11] with an intervening membrane allowing for calibration. Since precision at low pO_2 levels (<50 mm Hg) was desired, zero calibration of the sensor at 45°C was performed in 100% nitrogen. After calibration, the transcutaneous electrode was applied to skin adjacent to the exposure site; tcO_2 was monitored continuously during the experiment.

Topical 8-MOP Sensitization Fifty milliliters of 0.003% aqueous 8-MOP solution were introduced into a 3 cm-diameter cylinder held firmly to the skin of the inner forearm and left in place for 15 min. Irradiation of the treated site with UVA began within 7 min after application. This method results in reproducible photosensitization [12,13].

Procedure

Effect of Ischemia upon Pigment and Erythema Responses: In each subject the dose thresholds for the induction of erythema and pigment responses to UVA, UVB, and PUVA were examined. A geometric series of 30% increments of exposure dose were given to establish dose thresholds. Exposures were administered to the untanned hairless skin of the flexor surface of the forearm, to 8 circularly arranged sites 2 cm^2 in size. The sphygmomanometer cuff was then applied to the contralateral arm and inflated to the desired pressure as described above. Exposures were then administered to the ischemic skin of the forearm at a symmetrical location. The UVA dose range administered was adjusted if necessary to encompass the desired end point (pigment or erythema). Pigment responses were examined at 0 h, 24 h, and 7 days, erythema at 0 and 24 h. Erythema responses to PUVA were read at 72 h. Responses were graded as follows:

Trace	Minimum perceptible increase in erythema or pigmentation
+/−	Erythema or pigmentation clearly visible but not filling the exposure site
1+	Erythema or pigmentation filling the exposure site with well-defined borders
2+	More intense erythema or pigmentation

The dose threshold of UV resulting in 1+ pigmentation or 1+ erythema was defined as the minimum melanizing dose (MMD), the minimum erythema dose (MED), or the minimum phototoxic dose (MPD). In each individual the dose thresholds for erythema or pigmentation under conditions of normal blood flow were compared with the dose thresholds obtained at several different cuff pressures.

Transcutaneous Oxygen Administration: These experiments were performed on tape-stripped skin. Tape stripping was achieved by approximately 10 applications of adhesive tape (Blenderm) until a uniformly glistening surface was achieved. Exposures were administered through a quartz-roofed chamber 9 × 6 cm in size and 1 cm in depth, which could be perfused with oxygen, nitrogen, or air during exposure. Erythema responses to 75 J/cm^2 of UVA were studied in 5 Skin Type 1 or 2 individuals. Pigment responses to 60 J/cm^2 of UVA were examined in 5 Skin Type 3 or 4 individuals. In each case three 1 × 1 cm sites enclosed by the chamber were exposed under the following conditions:

1. Normal blood flow with the chamber containing air
2. Blood flow occluded (150 mm cuff pressure), chamber perfused with 100% nitrogen
3. Blood flow occluded (150 mm cuff pressure), chamber perfused with 100% oxygen.

Responses were graded as described above, at the same time points.

RESULTS

Effect of Cuff Inflation Upon Transcutaneous pO_2 Measurements Inflation of the cuff to 150 mm Hg, which exceeded systolic blood pressure in every individual, resulted in a rapid fall in the transcutaneous pO_2 measurement, from a mean of 75 mm Hg to less than 5 mm Hg in each subject. The minimum value was reached within 3.5 min of inflation, and remained steady during the period of the experiment. At intermediate cuff pressures between 0 and 150 mm Hg the pO_2 fell to an extent related to the cuff pressure as shown in Fig 1. Again the value equilibrated within 3.5 min of cuff inflation to each pressure.

Effect of Cuff Inflation upon Pigment and Erythema Responses to UVA A cuff pressure of 150 mm Hg prevented the development of the pigment response to UVA in every subject. Doses up to 10-fold greater than the MMD failed to elicit pigmentation at 0 h, 24 h, or 7 days. Intermediate cuff pressures caused an increase in the MMD which varied according to the cuff pressure as shown in Fig 2.

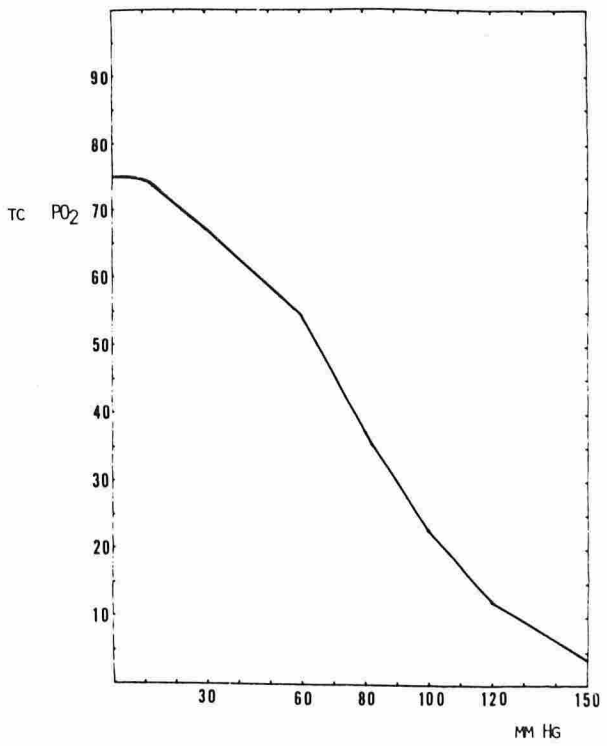

Figure 1. Transcutaneous oxygen tension ($tcPO_2$) (*mm Hg*) in the forearm following cuff inflation and a 5-min equilibrium period vs cuff inflation pressure.

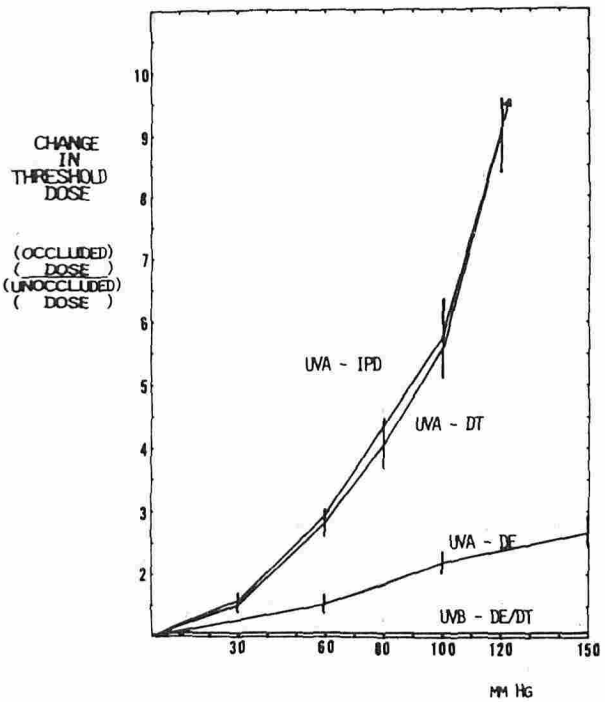

Figure 2. Change in dose threshold for the induction of pigmentation and erythema vs cuff inflation pressure. *IPD*, immediate pigment darkening; *DT*, delayed tanning; *DE*, delayed erythema (24 h).

Erythema responses were also inhibited by cuff inflation but to a lesser extent. At a cuff pressure of 150 mm Hg a mean increase in the MED of 2.7-fold was observed at 24 h. Immediate erythema could not be graded accurately after cuff application, because of reactive hyperemia following deflation.

Effects of Cuff Inflation upon Cutaneous Pigment and Erythema Responses to UVB and PUVA Neither pigment nor erythema dose thresholds of UVB or PUVA were altered by inflation of the cuff to 150 mm Hg.

Effect of Transcutaneous Oxygen Administration

Pigmentation: The results are displayed in Table I. Under normal blood flow conditions, each individual developed immediate and 7-day pigmentation responses to 60 J/cm^2 UVA administered through the quartz chamber. When blood flow was occluded and the chamber was perfused with nitrogen, no pigment responses were observed. When blood flow was occluded and the chamber was perfused with oxygen, responses were equal in each subject to those seen under conditions of normal blood flow.

Erythema: Exposure to 75 J/cm^2 UVA resulted in erythema 24 h later in each subject. Erythema responses were inhibited by ischemia when the chamber was perfused with nitrogen, but were fully restored in 3 of 5 subjects and partially restored in 2 of 5 subjects when oxygen was perfused through the chamber during exposure. These results are displayed in Table II.

Table I. Pigment Responses to 60 J/cm^2 UVA in Skin Types III and IV (Tape-Stripped Skin)

	Normal Blood Flow		Blood Flow Occluded + N_2		Blood Flow Occluded + O_2	
Subject	IPD	DT	IPD	DT	IPD	DT
1	+[a]	+	0	0	+	+
2	+	+	0	0	+	+
3	+/−	+/−	0	0	+/−	+
4	+	+	0	0	+	+
5	+	+	0	0	+	+

Key: IPD = immediate pigment darkening
DT = delayed tanning
[a]See "Procedure" under *Materials and Methods* for key to symbols.

Table II. Delayed Erythema Responses to 75 J/cm^2 UVA in Skin Types I and II (Tape-Stripped Skin)

Subject	Normal Blood Flow	Blood Flow Occluded + N_2	Blood Flow Occluded + O_2
1	+[a]	0	+
2	+	0	+/−
3	+	0	+/−
4	+	0	+
5	+	0	+

[a]See "Procedure" under *Materials and Methods* for key to symbols.

DISCUSSION

These results demonstrate fundamental differences between the mechanisms of cutaneous erythema and pigment responses to UVA and the responses to UVB exposure. Oxygen dependence of responses to UVA exposure have been demonstrated in other biologic systems [2–5] and distinguish these effects from those resulting from shorter wavelength exposure. Both immediate and 7-day pigment responses to UVA were inhibited by ischemia to an almost equal extent. UVB-induced delayed pigmentation was not affected. This is consistent with a relationship between UVA-induced pigmentation at early and late time points, and does not support the concept that the immediate pigment darkening (IPD) response is an independent oxidative process induced by UVA, while the tanning induced by UVA occurs by a separate pathway similar to that stimulated in the UVB-induced pigment response.

Reversal of the effects of ischemia by transcutaneous oxygen confirms that the effect is due to hypoxia rather than to other changes, such as alteration in pH, or depletion of a substrate. Transcutaneous oxygen was not effective unless tape-stripping was performed. This is not surprising since atmospheric oxygen might otherwise be expected to have diminished the effect of anoxia induced by cuff inflation upon pigment responses.

The UVA-induced erythema response was only partially inhibited by anoxia. The following is a hypothesis which could explain our observations: UV-evoked erythema may result from activation of 2 pathways, an oxygen-independent pathway for which the action spectrum lies in the UVC and UVB regions, and the photodynamic pathway with an action spectrum lying in the UVA region. The 2 action spectra overlap in the shorter UVA region. The sum of the 2 is the measured erythema action spectrum. The pigmentation action spectrum is similar in shape [14] and is also the result of addition of 2 action spectra as described for erythema, but longer wavelengths are relatively more effective at inducing pigmentation than erythema in genetically capable tanners [15] via an oxygen-dependent pathway. Consequently, for pigmentation, the contribution of the oxygen-independent (short wavelength) pathway to pigmentation induced by wavelengths greater than 320 nm is relatively small compared with its contribution to erythema. The contribution of the oxygen-independent pathway is sufficient to allow erythema to occur as a result of exposure to wavelengths greater than 320 mm when the dose is increased sufficiently. This hypothesis is testable using narrow-band exposure under anoxic and oxic conditions. However the intensity required to deliver appropriate doses during a period for which anoxia is practical would be very high. Peak et al [16] have proposed an analogous condition of "UVA mechanisms" and "UVC mechanisms" for mutagenesis and lethality in *Escherichia coli*, the action spectra overlapping mainly between 320–340 nm. The UVA mechanism is oxygen-dependent, and the 2 pathways make equal contributions to mutagenesis at 333 nm.

Cutaneous responses to PUVA were not inhibited by O_2 deprivation. This is consistent with previous studies showing that psoralen can sensitize by nonphotodynamic mechanisms [17–20]. It has been shown that phototoxicity of various psoralens correlates better with their ability to photobind with DNA than to produce $^1O_2$ [21], and exposure conditions that maximize cross-linking have been shown to result in greater cutaneous phototoxicity [22]. It is likely that more than one mechanism is involved in this mechanism. Opposing this conclusion is the observation that furocoumarins are effective photosensitizers for the generation of $^1O_2$ [23,24], which correlates with the ability of psoralens to induce erythema [25]; furthermore, quenchers of $^1O_2$ formation, e.g., beta-carotene, can inhibit erythema production by 8-MOP [26,27]. However, this effect could result from quenching of the triplet state of 8-MOP [28] by beta-carotene, thus preventing its cross-linking with DNA.

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