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A CLINICAL TRIAL OF THE EFFECTS OF ORAL BETA-CAROTENE ON THE RESPONSES OF HUMAN SKIN TO SOLAR RADIATION*

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ABSTRACT

Beta-carotene (180 mg/day, p.o.) or a placebo was administered to 30 normal male volunteers for 10 weeks, after which the volunteers were exposed to sunlight in the Arizona desert for up to 2 hours. Beta-carotene had a small but statistically significant effect in increasing the minimal erythema dose of sunburn radiation. The observed effects were too small to recommend the use of beta-carotene as a photoprotective agent for sunburn, but the methods developed provide a workable model for randomized controlled trials for evaluating the efficacy of systemic photoprotective agents.

Carotenemia induced by feeding beta-carotene ameliorates the photosensitivity reaction to visible light (380–500 nm) of patients with erythropoietic protoporphyria (1, 2). This finding, and two earlier reports that carotenemia prevented burns during heliotherapy (3) and facilitated tanning of the skin (4), led to a study to determine if orally administered beta-carotene would prevent or decrease the "sunburn" response to solar radiation (290–320 nm) of normal fair-skinned individuals with no unusual sensitivity to light.

A controlled clinical trial is presented and shows that high doses of orally administered beta-carotene have a small but statistically significant effect in increasing the minimal erythema dose for eliciting erythema produced by sunburn radiation.

MATERIALS AND METHODS

Study group. Adult male inmates of the Arizona State Prison in Florence, Arizona, ranging in age from 21 to 49 years old, volunteered for the study. Subjects were selected on the following criteria: a) fair skin, with or without some freckling that sunburns easily; b) no history of any photoallergic or phototoxic reactions, from either topical or systemic administration of photosensitizing compounds; and c) no history of porphyria or polymorphic light eruptions. All volunteers underwent physical examination, and the 30 ultimately chosen out of 70 were in good health. The volunteers were randomly assigned either to the group receiving carotene (C group) or to the group receiving placebo (P group). They took their respective medications for 10 weeks. The dose of beta-carotene was 180 mg per day contained in six capsules (Hoffmann-La Roche beta-carotene "beadlets"). The P group received six capsules daily of a placebo capsule. Both medications were administered by a code number, and the volunteers were informed that they were being tested for the comparative effect of two treatments. At the beginning of the study, prior to the administration of medication, and every 2 weeks until the completion of treatment, blood was drawn for hematological study, urea nitrogen, glucose, total bilirubin, glutamate-oxalacetate transaminase, carotene and vitamin A levels.

Evaluation of photoprotection by beta-carotene. Two separate determinations were undertaken to investigate the photoprotection against sunburn radiation: 1) determination of minimal erythema dose (MED) and 2) determination of the degree of sunburn (erythema) after exposure to 1 hour and 2 hours of solar radiation. During the early part of May in Florence, Arizona, these exposures are equivalent to approximately 6 and 12 times the MED for a fair-skinned individual. The MED is defined as the minimal dose of ultraviolet radiation (290–320 nm) that produces minimally perceptible redness at 24 hours after exposure.

Two templates were used on the backs of volunteers, and the dimensions and positioning of the templates are shown in Figure 1. The upper template (scapular and infrascapular regions) was used for the determination of the MED, and the lower template (infrascapular and lumbar regions) was used for the determination of the sunburn response to 60 and 120 minutes' exposure.

In order to randomize the order of exposure of the template apertures, the men were first divided, using a table of random numbers (5), into six groups of five men each, irrespective of whether the subjects were in the C or P groups. The apertures were exposed in the same order for all five men in each group. Then, by the use of random number tables, the order of exposure of the apertures was determined for both templates for each of the six groups. As an example, Figure 1 also shows the order of exposure of the template apertures of one of the groups.

The men were exposed to the sun from 11:30 a.m. to 1:30 p.m. on a cloudless day. The intensity of sunburn-producing ultraviolet radiation (290–320 nm) measured

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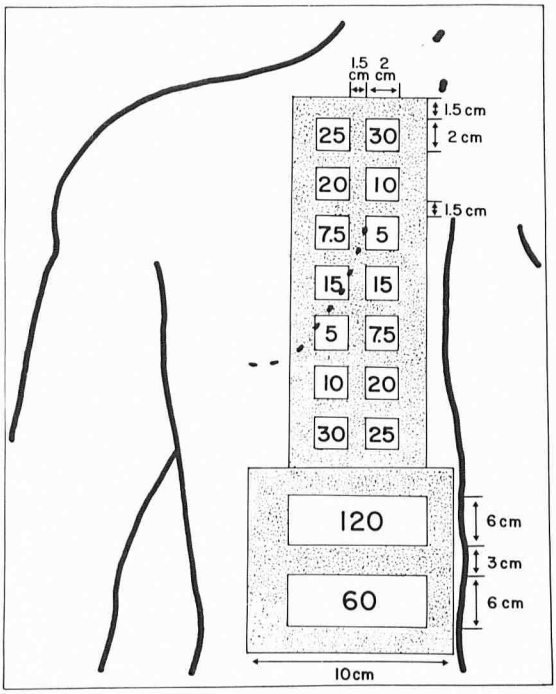

FIG. 1. Position of the templates on the back of each volunteer. The numbers in each aperture represent the minutes of exposure for each aperture, as used in one group of five volunteers. The order of exposure of the squares in the right-hand column is always the reverse of the order of exposure in the left-hand column. The apertures were uncovered as follows: 11:30 a.m., 120 minutes; 12:00 p.m., 60 minutes; 12:15 p.m., 30 minutes; 12:20 p.m., 25 minutes; 12:25 p.m., 20 minutes; 12:30 p.m., 15 minutes; 12:35 p.m., 10 minutes; 12:37.5 p.m., 7.5 minutes; 12:40 p.m., 5 minutes. At 12:45 p.m., all apertures exposed for 5–30 minutes were covered; at 1:00 p.m., the apertures exposed for 1 hour were covered; and at 1:30 p.m., the apertures exposed for 2 hours were covered.

at 12 noon on that day was approximately 6.5×10^2 $\mu w/cm^2/sec$. Prior to exposure, the templates were positioned on the subjects' backs, and the apertures covered with strips of tape numbered to correspond to the time of exposure. The remaining portions of the back not involved in the direct exposures were treated with a solution of 5% para-amino benzoic acid in 70% ethyl alcohol (PABA) (6) and then covered with black cloth to insure the availability of unexposed skin for repeat exposure if necessary. Because of the intense heat of the Arizona desert, other exposed areas of the body were not covered with cloth, but received only liberal application of the PABA solution. For the back, we used the double protection of PABA solution and black cloth because of the great intensity of the desert sunlight. Perhaps this double protection was unnecessary but no specific studies were undertaken of this point. With care in application there is no spread of the PABA. If, later, the treated side were needed for sun exposure experiments, showering would remove all of the PABA.

An open large concrete-paved court was used for exposure to the sun. Each subject lay prone on a cotton mattress during the exposure period. Care was taken to keep the subjects stationary in the prone position, and to allow the sun's rays to impinge directly on the back. To compensate for the changing angle of the sun's rays during the period of exposure, the subjects, while lying on the mattresses, were rotated clockwise through an angle of approximately 15° every 30 minutes. One investigator was in charge of monitoring the schedule of exposure for each group of five subjects. During the experimental period, the template apertures were exposed and covered in the order shown in the caption of Figure 1. The individuals in each group of five were treated in the same order at each exposure time.

Immediately after the period of exposure, the entire back was covered and the individuals returned inside. The templates were removed, and the skin was examined at that time, and again at 24, 48 and 96 hours after exposure, for the presence and degree of erythema, edema, pigmentation and desquamation. At 24 and 48 hours after exposure, two observers working independently of each other examined all the exposed sites. Immediately after exposure, and at 96 hours, only one observer made the examinations. The degree of erythema of each exposed site was evaluated subjectively as well as objectively. The subjective evaluation involved visual grading of erythema according to the criteria listed in Table I. For objective evaluation of erythema, the skin reflectance was measured with a Photovolt model 610 reflectance meter equipped with a green tristimulus filter with a maximum transmittance between 540–575 nm. The filter was placed in the reflectance-detecting probe, which contained a 25-watt tungsten bulb over a collimating lens and a photocell. Pure white magnesium carbonate was used to calibrate the meter to 100% reflectance. The percent skin reflectance readings of the control (unexposed skin adjacent to exposed site) and the exposed sites were obtained. The difference between these readings, the reflectometer difference (RD), was used as the objective indication of the amount of erythema. The degree of pigmentation was assessed according to the criteria listed in Table I.

RESULTS

Of the 30 volunteers, 18 were assigned to the C group and 12 to the P group, by use of the table of random numbers (5). One man from the C group was paroled two weeks before the end of the study, thus leaving 17 in the C group. Within a few weeks of carotene therapy, the blood carotene levels of all members of the group rose to several times the pre-treatment values (C-group range: 640–1360 $\mu g\%$; P-group range: 96–192 $\mu g\%$). The palms and soles of all the C group volunteers were colored but the skin of the rest of the body was only slightly yellower than the skin of the P group volunteers. No abnormal hematologic or chemical changes were noted with any of the tests used, and there was no other evidence of toxicity of beta-carotene. We plan to report in detail elsewhere the effects of induced carotenemia.

First, the reproducibility of visual grading of the presence and degree of erythema was determined. Two physicians, experienced in observing erythema, and working independently of each other, graded the presence or absence and degree of erythema of the exposed apertures at 24 hours after exposure of all individuals. The two physicians agreed in 95% of the cases as to the pres-

TABLE I

Criteria for the visual grading of erythema and pigmentation

Assigned grade*	Scale	Criteria for erythema	Criteria for pigmentation
0	0	No erythema	No change in skin color
1	±	Minimally perceptible erythema	Minimally perceptible pigmentation†
2	+	Definite erythema (pink) with well-defined borders	Light-brown pigmentation†
3	++	Marked erythema (red)	Moderate or medium-brown pigmentation†
4	+++	Fiery-red erythema without visible edema or tenderness	Dark-brown pigmentation with some residual erythema†
5	++++	Violaceous color accompanied by edema, blistering and tenderness	Intense dark-brown pigmentation without any erythema

* The whole-number notation was used in the statistical analyses.

† Pigmentation accompanied by erythema observed between 72–120 hours after exposure to sun can best be assessed by the examination of the exposed sites after blanching.

ence or absence of erythema in both the C and P groups. They agreed with respect to degree of erythema in 85% of the observations in the C group and in 77% of the observations in the P group. This difference in agreement between the C and P groups was found not to be statistically significant. Thus, the presence of and degree of erythema can be reproducibly observed. In the data given below, the findings of the observers were therefore averaged.

There was good agreement between visual grading of the erythema and the measurement by reflectometer in both the C and P groups (Fig. 2), as earlier noted by Daniels (7, 8). The slight difference between the slopes of the lines for the C and P groups was not significant (t-test for difference in slopes). This indicates that although all volunteers in the C group were carotenemic at the time of light testing, the slight yellow color of the skin of their backs did not interefere with either visual or reflectometer grading of erythema.

As illustrated in Figure 1, the upper template consists of two parallel columns of squares; the exposures of the left-hand column are repeated in the reverse order in the right-hand column. There was no significant difference in the occurrence of or degree of erythema between the columns, so each column was treated as a separate reading, giving 34 readings in the C group and 24 readings in the P group.

Figure 3 shows the minimal time of exposure to the sun required to produce barely perceptible erythema at 24 hours after exposure for each subject in each of the two groups. The data are presented as the frequency distribution (number of subjects developing erythema at 24 hours at each exposure time) and the cumulative distribution. The difference was significant between the C and the P groups for the cumulative distribution (Kolmogorov-Smirnov test, $p < .025$), as well as for the frequency distribution (chi^2 test: $p < .01$) of first appearance of erythema at 24 hours.

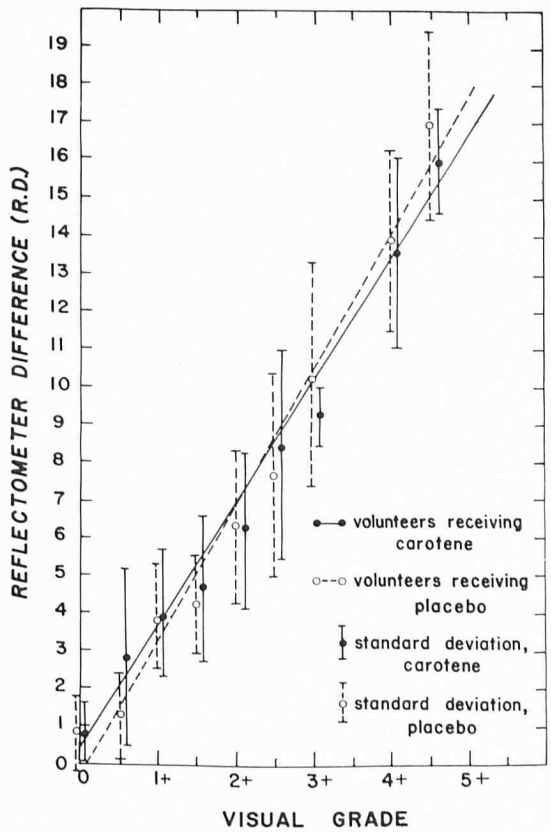

FIG. 2. Relation of visual grade to reflectometer difference (R.D.) of erythema observed at 24 hours in the carotene and placebo groups. The correlation lines for each group were obtained by the method of least squares.

There was no significant difference between the C and the P groups in the intensity of erythema after it developed whether graded by eye or by reflectometer (Table II). In addition, the mean grades of erythema, measured visually and by re-

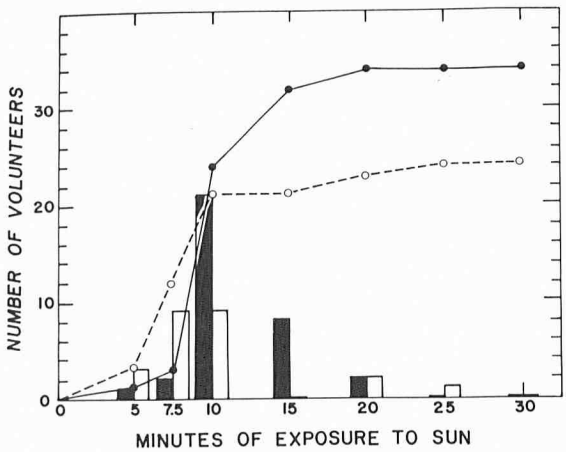

FIG. 3. Effect of induced carotenemia on the least time of exposure to sun required to produce minimally perceptible erythema. The solid boxes show the number of individuals in the carotene group developing minimally perceptible erythema at each exposure time; the open boxes show the number of individuals in the placebo group developing minimally perceptible erythema at each exposure time. ●——● cumulative distribution, carotene group; O- - -O cumulative distribution, placebo group.

TABLE II

Visual and reflectometer grading of erythema in the carotene and placebo groups

Exposure to sun (minutes)	Erythema			
	Mean visual grade*		Mean reflectometer difference†	
	Carotene group	Placebo group	Carotene group	Placebo group
5	.015	.083	.765	1.208
7.5	.103	.417	.882	2.000
10	.647	.812	2.912	3.083
15	.912	1.187	3.500	3.833
20	1.544	1.541	5.176	4.375
25	1.949	1.791	5.853	5.708
30	2.228	2.082	7.500	7.250
60	3.882	3.604	12.529	12.417
120	4.000	4.125	14.471	14.500

* Sum of all observations at each time divided by the number of observations in the respective group. Grading scale was from 1+ to 5+; and where the two observers differed, the average reading was taken.

† Sum of all reflectometer differences at each time divided by the number of men in each group.

flectometer, were plotted against the various exposure times, but no significant difference was observed between the C and the P groups ($p < .2 > .1$).

No significant difference between the C and P groups was found in either the appearance of or degree of erythema or edema in the areas of skin exposed for 60 and 120 minutes as graded by eye or by reflectometer (Table II). On the other hand, at 96 hours after exposure, the areas exposed for

TABLE III

Number of men developing pigmentation in both groups, and mean grade of pigmentation after exposure to the sun for 60 and 120 minutes

Minutes of exposure to sun	Carotene group		Placebo group	
	Number of men developing pigmentation*	Mean grade of pigmentation	Number of men developing pigmentation	Mean grade of pigmentation
60	17/17†	2.35‡	8/12†	1.50‡
120	17/17	2.88	10/12	2.25

* Pigmentation was observed at 96 hours after sun exposure.

† $p < .025$.

‡ $p < .05$.

60 and 120 minutes of the C group volunteers were more pigmented than the corresponding areas of the P group ($p < .025$ for 60 minutes and $p < .1 > .05$ for 120 minutes' exposure) (Table III). In addition, the number of men in the C group developing pigmentation after 60 minutes of exposure to the sun was significantly greater than the number of those in the P group who developed pigmentation ($p < .025$) (Table III). No desquamation was observed in any of the volunteers.

DISCUSSION

Although induced carotenemia did not significantly alter the degree of erythema, the threshold dose of sunlight to produce erythema (MED) was significantly greater in carotenemic individuals. However, this effect is too small to justify recommending beta-carotene as a general photoprotective agent against sunburn radiation. An additional effect was the tendency of those receiving beta-carotene to manifest more pigmentation (tanning) after exposure to sunlight. This latter observation is consistent with the data of Bendes and Sandler (3, 4) in studies that were less rigidly controlled. Histologic studies were not performed on the sun-exposed skin from the carotenemic individuals. Therefore we cannot estimate the degree of epidermal cell damage that was produced after exposure to the sun, and that presumably was ameliorated by carotenemia.

Beta-carotene in the doses used in this study protects patients with erythropoietic protoporphyria against photosensitivity to visible light (1, 2), but it has only a small effect in protecting against sunburn radiation. This suggests that the molecular mechanisms for reactivity of the skin to visible light and to sunburn radiation may be different, and that beta-carotene is effective in ameliorating only certain photosensitivity reactions.

In studies of efficacy of drugs, double-blind designs are useful to minimize bias. When double-blind designs are not feasible, as in the present study in which the compound under investigation

caused a recognizable change in the appearance of the skin, detailed attention must be paid to randomization and to the elimination of observer bias. We have presented here a workable model for the evaluation of the effects of systemic photoprotective agents.

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