TREATMENT OF LIGHT SENSITIVITY WITH CAROTENOIDS

Serum Concentrations and Light Protection

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Abstract. When beta-carotene was administered to 24 volunteers as single oral doses varying from 2 to 8 mg per kg bodyweight, the average maximal increase of carotenoid concentration in serum was about 30 µg per 100 ml, though with great individual variations. Maximal serum concentrations were obtained mostly within 4 hours after the administration. The binding of beta-carotene to human serum proteins was estimated to about 75%, by equilibrium dialysis.

Carotenoids were then given as a combination of beta-carotene and canthaxanthin, in multiple oral doses of 50 mg every twelve hours, to patients with light-sensitive psoriasis, polymorphous light eruptions and erythropoietic protoporphyria, but also to a control group consisting of not particularly light-sensitive patients with vitiligo. Maximal serum concentrations of carotenoids were reached after treatment for 10 to 40 days by most patients, with the fastest increase during the first 15 days. The maximal serum concentrations ranged from 338 to 1 219 µg per 100 ml, and the average for these two groups of patients was about the same.

The maximal light protection factor for the group of patients with light sensitivity varied between a factor of 4 and 8, with an average value of 5.4. In the control group of patients with vitiligo the average light protection factor was 1.6 for normal, and 1.9 for vitiliginous skin. According to results obtained by light testing at various time points during the carotenoid treatment, the maximal protection factor was never estimated before about 2 to 4 months of treatment. For patients with polymorphous light eruptions there is a positive correlation between maximal light protection and maximal serum concentration ($r = 0.74$), but none for the patients in the control group. In this study a pronounced difference in the light protection factor could be distinguished for light-sensitive patients vis-a-vis a control group, in spite of the fact that the minimal erythema dose before treatment, whereas the serum concentration obtained during treatment was approximately the same for both these groups.

There are several earlier reports concerning the absorption and metabolism of beta-carotene; most of the them emphasize the relationship with vitamin A (5, 12, 14, 20, 25, 26). There has also been a great interest in carotenoids, stressing their light-protective mechanisms, in particular in microorganisms and plants (17, 23, 24, 29, 30, 31, 39).

Good therapeutic effects have been obtained with carotenoids in patients with pronounced light sensitivity, such as in erythropoietic protoporphyria (2, 7, 27, 32, 33) and polymorphous light eruptions (35, 40). Work has also been done to elucidate the mechanisms underlying the light-protective effect (1, 4, 6, 8, 9, 10, 11, 23, 24, 41).

Light protection obtained by beta-carotene slowly increases with time after the inception of therapy, and the maximal effect is reached after several weeks of treatment. The purpose of the present study is to give more information on the time course of carotenoid concentrations in serum, when given to fasting subjects as single or multiple oral doses, and to see whether or not there is any correlation between serum concentration and light protection.

As shown by earlier experiments, the absorption of carotenoids is dependent on test meal regimens (5, 12, 14, 20, 25, 26). Therefore, the present study with single oral doses was confined to fasting individuals but with different formulations, capsule and water suspensions, and with varying doses of beta-carotene.

In a group of patients treated with carotenoids for light sensitivity, the serum concentrations and the increase of light tolerance was observed. As a control group, patients with vitiligo but otherwise normal light sensitivity were used, as they offered both normally pigmented skin, and unpigmented spots. It is known that carotenoids combine with proteins to form carotenoproteins (4, 13, 43). This phenomenon has been extensively studied by various authors in human serum too (5, 22). The concentration dependence of the binding of beta-carotene to human serum proteins was investigated in the present study by equilibrium dialysis.
MATERIAL AND METHODS

Determination of total carotenoids in human serum

The serum concentrations of carotenoids were analysed by colorimetric measurement (3, 5). Freshly drawn blood from patients or volunteers was centrifuged at 3,000 rpm for 15 min. 1.0 ml serum was then transferred to another tube and 1.0 ml of absolute ethanol added drop by drop and mixed thoroughly for denaturation. Carotenoids were then extracted by adding 2.0 ml of n-hexane and shaken for 10 min.

The solution was centrifuged and the n-hexane layer immediately used for the colorimetric measurement. 1.3 ml of the n-hexane layer was pipetted into a semi-micro cell of 1 cm light path and the absorbance at 454 nm was read on a Beckman DB spectrophotometer against n-hexane as a blank.

A calibration factor was determined by measuring the absorbance of crystalline beta-carotene dissolved in hexane in known concentrations. The concentration of carotenoids in serum was calculated from the following formula:

Absorbance = 750 - \( \mu g \) of carotene per 100 ml of serum.

The serum samples were processed regularly in duplicates and, when possible, in triplicates.

The reproducibility of the method was determined for 20 analyses of a pooled serum from patients on long-term treatment with carotenoids, and the mean was 994 \( \mu g \) per 100 ml and the standard deviation 12 \( \mu g \) per 100 ml.

This method measures the total carotene concentration in human serum. The normal range without carotene treatment is 60 to 200 \( \mu g \) per 100 ml (28).

Protein binding

The degree of protein binding was determined by equilibrium dialysis using cellophane dialysis tubing. Each bag contained 10.0 ml pooled serum with known concentrations of carotenoids. The dialysis was performed in a bath of slowly running water. The equilibrium concentrations of carotenoids were determined spectrophotometrically as described above. Several different dialysis periods were used, viz. 1, 2, 4, 6, 8, 16 and 24 hours. The initial serum concentrations of beta-carotene before dialysis were varied from 50 to 1,125 \( \mu g \) per 100 ml in 5 samples.

Administration of carotenoids

Pure beta-carotene, obtained from Hoffman-La Roche Ltd., was administered orally in gelatin capsules to 20 healthy volunteers, in a single dose of 2, 3, 4 or 6 mg per kg body weight. To 4 further volunteers beta-carotene was dispensed as a water solution in a dose of 8 mg per kg body weight. The single dose was always taken in the morning and the subjects were fasting. Two subjects (G. W. and H. S.) were given a single oral dose on two different occasions, first dispensed as a water solution and secondly as gelatin capsules.

Multiple oral doses of carotenoids were given as capsules to 12 patients with various light dermatoses (Table II). 8 patients were treated for polymorphous light eruptions (PMLE), 3 patients for psoriasis with light sensitivity and 1 patient for erythropoietic protoporphyria (EPP). It should be noted that the patients H. K., B. M., R. B., V. H., M. S. V. and M. B. B. in Table II showed a pronounced sensitivity to longwave ultraviolet light (UVA) as revealed by light testing.

RESULTS

Single-dose experiments

The initial fasting values of serum carotenoids, before administration of beta-carotene, ranged from 23 to 184 \( \mu g \) per 100 ml for the 24 investigated healthy volunteers. The mean value was 80 \( \mu g \), with a standard deviation of 37 \( \mu g \).

Table I summarizes data for the different dose groups. As there were great individual variations of the caroteneoid fasting values in serum, the increase in carotenoids were not expressed in percentages but in absolute values. The increment in absolute units, expressed as \( \mu g \) per 100 ml serum, was about the same for the different groups. This as a control group of not particularly light-sensitive patients, 10 healthy individuals with vitiligo were given multiple oral doses of carotenoids. One of these subjects (E. B.) had a total confluent vitiligo.

Two of the patients with vitiligo (E. B. and B. E. M.) were given pure beta-carotene in a dose of 20 mg every 12th hour. Every second patient on multiple oral dose received capsules which contained a combination of 10 mg beta-carotene and 15 mg canthaxanthin each, in a total dose of 50 mg every 12th hour. The treatment was continued for 2 to about 4 months; each single dose corresponded to about 0.5 to 1.0 mg per kg body weight.
Table I. Results of single-dose administration of pure beta-carotene in doses between 2 and 8 mg/kg bodyweight

Patients 21 to 24 were given beta-carotene as a water solution.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Bodyweight</th>
<th>Dose (mg/kg)</th>
<th>Concentrations of carotenoids/serum (µg/100 ml)</th>
<th>Hours for maximal increase</th>
<th>Maximal carotenoid increment (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before treatment</td>
<td>Maximal value</td>
<td></td>
</tr>
<tr>
<td>1. EVA</td>
<td>♂</td>
<td>80</td>
<td>2</td>
<td>79</td>
<td>131</td>
<td>2</td>
</tr>
<tr>
<td>2. AL</td>
<td>♂</td>
<td>84</td>
<td>2</td>
<td>75</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>3. RH</td>
<td>♂</td>
<td>75</td>
<td>2</td>
<td>184</td>
<td>218</td>
<td>1</td>
</tr>
<tr>
<td>4. SN</td>
<td>♂</td>
<td>105</td>
<td>2</td>
<td>45</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>5. HS</td>
<td>♂</td>
<td>74</td>
<td>2</td>
<td>47</td>
<td>61</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. AB</td>
<td>♂</td>
<td>64</td>
<td>3</td>
<td>68</td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td>7. MF</td>
<td>♂</td>
<td>62</td>
<td>3</td>
<td>23</td>
<td>45</td>
<td>4</td>
</tr>
<tr>
<td>8. OL</td>
<td>♂</td>
<td>70</td>
<td>3</td>
<td>139</td>
<td>180</td>
<td>4</td>
</tr>
<tr>
<td>9. JC</td>
<td>♂</td>
<td>71</td>
<td>3</td>
<td>79</td>
<td>113</td>
<td>4</td>
</tr>
<tr>
<td>10. PB</td>
<td>♂</td>
<td>67</td>
<td>3</td>
<td>38</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. KL</td>
<td>♂</td>
<td>53</td>
<td>4</td>
<td>71</td>
<td>79</td>
<td>4</td>
</tr>
<tr>
<td>12. NJ</td>
<td>♂</td>
<td>73</td>
<td>4</td>
<td>107</td>
<td>127</td>
<td>8</td>
</tr>
<tr>
<td>13. TL</td>
<td>♂</td>
<td>83</td>
<td>4</td>
<td>23</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>14. SL</td>
<td>♂</td>
<td>80</td>
<td>4</td>
<td>103</td>
<td>144</td>
<td>8</td>
</tr>
<tr>
<td>15. GW</td>
<td>♂</td>
<td>72</td>
<td>4</td>
<td>110</td>
<td>146</td>
<td>8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. IN</td>
<td>♂</td>
<td>53</td>
<td>6</td>
<td>125</td>
<td>144</td>
<td>2</td>
</tr>
<tr>
<td>17. AE</td>
<td>♂</td>
<td>63</td>
<td>6</td>
<td>67</td>
<td>94</td>
<td>2</td>
</tr>
<tr>
<td>18. BJ</td>
<td>♂</td>
<td>55</td>
<td>6</td>
<td>110</td>
<td>134</td>
<td>2</td>
</tr>
<tr>
<td>19. KK</td>
<td>♂</td>
<td>63</td>
<td>6</td>
<td>70</td>
<td>107</td>
<td>4</td>
</tr>
<tr>
<td>20. SE</td>
<td>♂</td>
<td>72</td>
<td>6</td>
<td>41</td>
<td>70</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21. UL</td>
<td>♂</td>
<td>60</td>
<td>8</td>
<td>94</td>
<td>169</td>
<td>4</td>
</tr>
<tr>
<td>22. IE</td>
<td>♂</td>
<td>60</td>
<td>8</td>
<td>75</td>
<td>94</td>
<td>1</td>
</tr>
<tr>
<td>23. GW</td>
<td>♂</td>
<td>72</td>
<td>8</td>
<td>94</td>
<td>113</td>
<td>2</td>
</tr>
<tr>
<td>24. HS</td>
<td>♂</td>
<td>74</td>
<td>8</td>
<td>51</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table II. Light-sensitive patients treated with multiple oral doses of carotenoids

Maximal values of carotenoids in serum and maximal light protection factor, expressed as increase of the minimal erythema dose in seconds. 8 patients had polymorphous light eruptions (PMLE), 1 patient erythropoietic protoporphyria (EPP) and 3 patients light-sensitive psoriasis (Psor). UVA indicates light testing with longwave ultraviolet light alone.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Diagnosis</th>
<th>Maximal value carotenoids/serum (µg/100 ml)</th>
<th>Minimal erythema dose, in seconds</th>
<th>Maximal light protection factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. BMO</td>
<td>PMLE</td>
<td>731</td>
<td>Before treatment: 54 After treatment: 324</td>
<td>6</td>
</tr>
<tr>
<td>2. HL</td>
<td>PMLE</td>
<td>468</td>
<td>Before treatment: 24 After treatment: 96</td>
<td>4</td>
</tr>
<tr>
<td>3. IP</td>
<td>PMLE</td>
<td>900</td>
<td>Before treatment: 30 After treatment: 120</td>
<td>4</td>
</tr>
<tr>
<td>4. MS</td>
<td>PMLE</td>
<td>1199</td>
<td>Before treatment: 10 After treatment: 80</td>
<td>8</td>
</tr>
<tr>
<td>5. EJ</td>
<td>PMLE</td>
<td>581</td>
<td>Before treatment: 20 After treatment: 100</td>
<td>5</td>
</tr>
<tr>
<td>6. HK</td>
<td>PMLE</td>
<td>788</td>
<td>Before treatment: 1 After treatment: 6</td>
<td>6</td>
</tr>
<tr>
<td>7. BM</td>
<td>PMLE</td>
<td>900</td>
<td>Before treatment: 4 After treatment: 28</td>
<td>7</td>
</tr>
<tr>
<td>8. KS</td>
<td>PMLE</td>
<td>544</td>
<td>Before treatment: 6 After treatment: 30</td>
<td>5</td>
</tr>
<tr>
<td>9. RB</td>
<td>EPP</td>
<td>1219</td>
<td>Before treatment: 120 (UVA) After treatment: 480 (UVA)</td>
<td>4</td>
</tr>
<tr>
<td>10. VH</td>
<td>Psor</td>
<td>650</td>
<td>Before treatment: 6 After treatment: 36</td>
<td>6</td>
</tr>
<tr>
<td>11. MSV</td>
<td>Psor</td>
<td>806</td>
<td>Before treatment: 6 After treatment: 30</td>
<td>5</td>
</tr>
<tr>
<td>12. MBB</td>
<td>Psor</td>
<td>506</td>
<td>Before treatment: 10 After treatment: 50</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>776.5</td>
<td>After treatment: 40</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* These patients were also sensitive for longwave ultraviolet light but the light testing was done with unfiltered Xenon light.

The maximal serum concentrations were reached after treatment for 20 to 40 days by 7 patients, and remaining patients showed a further (slight) progressive increase. There was a faster increase of the serum concentrations during the first 15 days, whereas the subsequent increase was usually much slower. When the maximal serum concentration was reached, a slight but successive decrease in the serum concentrations of 7 patients seemed to occur.

The maximal serum concentration obtained ranged from 338 to 1219 µg per 100 ml, and the initial carotenoid values before treatment ranged from 19 to 131 µg per 100 ml, with an average of 74.9 µg, and with no apparent correlation to the maximal serum concentration obtained.

The patients given pure beta-carotene started with a serum concentration within the above-mentioned range and reached a maximal value of 731 and 1106 µg per 100 ml, respectively.

**Protein binding**

When the protein binding of pure beta-carotene to human serum proteins was determined, the equilibrium state was reached within 8 hours. For
all 5 samples with different initial concentrations of beta-carotene, ranging from 50 to 1125 µg per 100 ml, a protein binding of about 75% was found. There seemed to be no correlation between initial carotenoid concentration and the degree of protein binding.

**Serum concentration and light tolerance**

The maximal serum concentrations of carotenoids were most regularly obtained after treatment for 20 to 40 days (Fig. 2). To obtain information on the most suitable time for light testing of the patients after commencement of the carotenoid treatment, a series of 6 patients were light tested at various time intervals. Fig. 3 gives the protection factor and serum concentration of carotenoids obtained by these patients, as a function of the duration of treatment. On the basis of these results we decided to light test the patients not earlier than after 2 months of treatment. In the following, the maximal protection factor has been determined after about 2 to 4 months of treatment.

In the group of light-sensitive patients (Table II), the maximal serum concentrations obtained varied from 468 to 1219 µg per 100 ml, with a mean value of 776.5 µg. The light protection factor for these patients varied between 4 and 8, with an average value of 5.4. In Fig. 4 the maximal light protection for these patients is plotted against the maximal serum concentration obtained, and for the patients with polymorphous light eruptions there seems to be a moderate correlation, with a correlation factor of 0.74.

In the control group of patients with normal light...
sensitivity of non-vitiliginous skin (Table III), the maximal serum concentrations ranged from 338 to 1 144 µg per 100 ml, with a mean value of 818 µg. The increase of light tolerance on normal pigmented skin and on vitiligo spots is given in Table III. The average light protection factor in this group was 1.6 for normal, and 1.9 for vitiliginous skin and never exceeded a factor of 3. The increase in minimal erythema dose after long-term administration of carotenoids in this group was thus slight, but just statistically significant ($p < 0.05$). As seen in Fig. 4, there seems to be no correlation between the maximal serum concentration and the protection factor obtained for these patients.

Thus the increase of carotenoids in serum was about the same for both these groups, but a pronounced difference in their light protection factor could be established (Fig. 4), in spite of the fact that their minimal erythema dose values before treatment were approximately the same.

All patients obtained a moderate yellow pigmentation, most pronounced on light-exposed areas and on the palms and soles, but also seen on vitiligo spots. Whereas normally pigmented skin also became darker, the contrasts remained and for the patients with vitiligo a cosmetic success was achieved only in the patient with total, confluent vitiligo.

### DISCUSSION

It is a clinical fact that in patients treated with carotenoids the degree of light protection increases slowly (2, 7, 32, 33, 40). The purpose of the present study was to obtain more information about the dependence of the light protection on the serum concentration of the carotenoid and on the length of the treatment period.

Single oral doses of beta-carotene gave a small increase of the serum concentration, which seemed...
to be limited to about 30 µg per 100 ml for all the different doses given. This is in agreement with earlier findings where a limited absorption capacity of the small intestine for carotene has been found (5, 12, 14, 20).

The time taken for the serum concentration of carotenoids to decrease from its maximal value to 50% of the maximal increment value, ranged from 26 to more than 96 hours. There seemed to be no correlation with the dose given. The variations seen were probably partly dependent on uncontrolled food intake, even though the subjects participating were asked to keep a diet as free from carotenoids as possible (3, 15, 16, 18, 44). However, the elimination of carotenoids from the blood seems to be so slow that a cumulative effect will occur if single doses of sufficient size are given daily.

Multiple-dose administrations of carotenoids gave fairly high serum concentrations, but maximal values were obtained first after 20 to 40 days of treatment. The increase in light protection was developed at the same time or perhaps somewhat later than the increase in serum concentration of carotenoids, as is seen in Fig. 3. There might be a lag between the increase in serum concentration and tissue concentration of carotenoids. From these results we therefore conclude that the light-protective effect of carotenoids on a photodermatosis should be tested after more than 2 months of daily oral intake. As there is normally a certain caroteneid concentration in human serum, there was no sense in estimating the degree of protein binding after single-dose experiments, but when measured after long-term treatment the protein binding was about 75%, irrespective of which level the serum concentration had reached.

Earlier findings indicate that patients with some types of light-sensitive diseases (2, 7, 19, 32, 33, 35, 40) have a much better light protection from beta-carotene than have patients with other photodermatoses (21) or normal individuals (34). In the present investigation this has been confirmed. In a group of vitiligo patients beta-carotene gave a light protection factor of 3 or less and, interestingly enough, there seemed to be no difference between vitiligo spots and normal skin with regard to light sensitivity, either before or after carotenoid treatment in a slightly pigmented Swedish population. These patients obtained the same serum concentration of carotenoids as did the light-sensitive group.

The light protection afforded by carotenoid treatment in 8 patients with PMLE, in 3 light-sensitive patients with psoriasis, and in 1 patient with EPP, was considerably better than in the control group. In these cases the light protection factor was between 4 and 8. For the patients with PMLE there seems to be a correlation between serum concentration of carotenoids and light protection (correlation coefficient −0.74).

Beta-carotene functions as a singlet oxygen quencher under certain circumstances (1, 6, 8, 9, 10, 11, 23, 24). We have reason to believe that this may be the case in biological systems too (41). It is tempting to speculate that the low protective effect of beta-carotene in normal individuals in spite of a high serum concentration indicates that singlet oxygen is not involved in the UV-erythema reaction of normal individuals but that in the case of PMLE and EPP, singlet oxygen may be involved. With regard to the PMLE patients there is definitely a correlation between the serum concentration of carotenoids and the light protection factor, though for normal and vitiliginous skin there is no evident correlation (r = 0.01 and 0.13 respectively).

For EPP it is likely that the photo-oxidation is sensitized by protoporphyrin and mediated by singlet oxygen (36, 37, 38). For PMLE we do not know which substance is the sensitizer. There is no indication that porphyrins are involved in the light reaction in PMLE. Another type of endogenous metabolite that has photohemolytic activity mediated by singlet oxygen is kynurenic acid (42). As yet it remains to be investigated whether kynurenic acid is the most important photosensitizer in PMLE.

In our photohemolysis studies we have found that for protoporphyrin-sensitized photohemolysis, the main inhibitory effect of beta-carotene is due to a quenching effect and not to a light filter effect (41).

What is puzzling is that some light-sensitive patients, such as those with PMLE, have a normal MED and get a considerably higher MED than normal individuals after carotenoid treatment (34, 40). We believe that the normal MED for the PMLE patients may be a question of dose rate, a factor that has been insufficiently investigated.

On the basis of our studies we are inclined to believe that the normal solar erythema reaction is not mediated by singlet oxygen and is therefore only slightly affected by carotenoids while the reaction in some photodermatoses, such as in EPP and PMLE, singlet oxygen is involved. It is even possible that when a photosensitizing metabolite is present...
in high concentrations another type of photochemical reaction is competing with the normal erythema-producing mechanism and therefore, under certain circumstances, a higher MED may be the effect while the patient develops cutaneous lesion by a relatively small repeated light dose.

Concomitant with the erythema reaction there may be damage to other cellular structures in the case of some photodermatoses. For diagnostic purposes, repeated light provocation is a better method than simply measuring MED.

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REFERENCES


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