Introduction

Epidemiological studies revealed that an increased consumption of fruits and vegetables is associated with a decreased risk of the development of several kinds of cancer. A positive correlation was also described for elevated β-carotene plasma levels and a diminished risk for lung and stomach cancer (1,2). However, intervention trials with β-carotene failed to prevent the development of lung cancers in Finnish smokers (3). It has been suggested that carotenoids other than β-carotene might at least in part be responsible for the protective effects. Several studies have demonstrated that non-provitamin A carotenoids such as lycopene or canthaxanthin also exhibit antioxidant activities of carotenoids have been considered as biochemical mechanisms underlying the cancer-preventive properties of these compounds. β-Carotene and other carotenoids, including those lacking provitamin A activity, proved to be active in both these parameters. The β-carotene analogs retrodehydro-β-carotene, echinenone, cryptoxanthin (3-hydroxy-β-carotene) and canthaxanthin stimulate GJC and efficiently deactivate singlet molecular oxygen. β-Carotene is less active than its retro-dehydro analog with respect to $^{1}\text{O}_2$ quenching but GJC is similar. The five-membered ring analog of canthaxanthin, dinor-canthaxanthin, has less effect on GJC as compared with the parent compound but exhibits increased singlet oxygen quenching. Straight-chain polyene dialdehydes are quenchers of singlet oxygen, the efficiency increasing with the number of conjugated double bonds. However, none of these compounds significantly induce GJC. These data indicate that the two properties of carotenoids addressed in this study may operate independent of each other.

Materials and methods

Chemicals

Lucifer yellow CH was obtained from Sigma (Deisenhofen, Germany). Canthaxanthan was a kind gift from Dr J.Braus, Hoffmann-La Roche (Basel, Switzerland). Retrodehydro-β-carotene and the dialdehydes were provided by Dr Paust, BASF (Ludwigshafen, Germany). The other compounds were synthesized according to published methods (14). All other chemicals were purchased from Merck (Darmstadt, Germany).

Aldehydes used were C(20)-dialdehyde: 2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptadecane-1,16-dial; C(30)-dialdehyde: 2,6,10,15,19,23-hexamethyl-tetracosane-2,4,6,8,10,12,14,16,18,20,22-undeca-1,24-dial; C(40)-dialdehyde: 2,6,10,14,19,23,27,31-octamethyl-diotracontane-2,4,6,8,10,12,14,16,18,20,22,24,26,28,30-pentacosane-1,31-dial.

Cell culture and growth conditions

The murine embryo fibroblast cell line C3H/10T1/2 clone 8 (ATCC No. CCL 226) was cultured in fibroblast growth medium FGM obtained from PromoCell (Heidelberg, Germany) and supplemented with 10% fetal calf serum from Life Technologies (Eggenstein, Germany) in 35×10 mm dishes from Nunc (Wiesbaden, Germany). The FCS content was decreased to 3% when cells were confluent and cells then were exposed to 10 μM of the respective carotenoid. The activity of capsorubin in the cell communication assay was tested in 1 μM concentration, because higher levels of this carotenoid proved to be toxic. Carotenoids were dissolved in tetrahydrofuran, stabilized with 250 p.p.m. of 2,6-di-tert-buty-4-methyl phenol by the manufacturer; final concentration of the solvent in cell culture was 0.5% (13). Controls received tetrahydrofuran 0.5%.

Abbreviation: GJC, gap junctional communication.

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Table I. Induction of cell–cell-communication in C3H/10T1/2 mouse fibroblasts by various carotenoids measured in the dye transfer assay compared with the quenching rate constants for singlet oxygen quenching as well as in vitro antioxidant capacity

<table>
<thead>
<tr>
<th>Compound</th>
<th>GJC (% of control)</th>
<th>(^1)O(_2) quenching rate constant (10^9) (M/s)</th>
<th>TEAC(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(^b)</td>
<td>100 ± 15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Six-membered ring system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>530 ± 21</td>
<td>8</td>
<td>1.9</td>
</tr>
<tr>
<td>Echinene</td>
<td>409 ± 27</td>
<td>9</td>
<td>0.7</td>
</tr>
<tr>
<td>Canthaxanthin</td>
<td>428 ± 24</td>
<td>9</td>
<td>0.02</td>
</tr>
<tr>
<td>4-OH-β-carotene</td>
<td>388 ± 30</td>
<td>9</td>
<td>2.0</td>
</tr>
<tr>
<td>Cryptoxanthin (3-OH-β-carotene)</td>
<td>307 ± 24</td>
<td>9</td>
<td>0.02</td>
</tr>
<tr>
<td>Retrodehydro-β-carotene</td>
<td>522 ± 32</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Five-membered ring system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsorubin</td>
<td>107 ± 11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Dinor-canthaxanthin</td>
<td>183 ± 23</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Violerythrin</td>
<td>148 ± 21</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Polyene chain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-20-Dialdehyde</td>
<td>120 ± 17</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>C-30-Dialdehyde</td>
<td>113 ± 14</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>C-40-Dialdehyde</td>
<td>125 ± 16</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)GJC was measured on day 5 after incubation with the respective carotenoid (10 \(\mu\)M); capsorubin was used in 1 \(\mu\)M concentration. Data are given as means ± SD (n = 10).

\(^b\)TAEC: Trolox equivalent antioxidant capacity; taken from reference (20).

\(^c\)Control was 0.5% tetrahydrofuran.

**Gap junctional communication assay**

On days 3, 5, 10 and 15 of the incubation, GJC was measured by microinjection of the fluorescent dye Lucifer yellow (10% in 0.33 M LiCl) into cells by means of a micromanipulator and a microinjection system (Eppendorf, Hamburg). The number of fluorescent cells around the injected cell was scored 5 min after injection and serves as an index of GJC (15). The data given in Table I and Figure 2 are mean values (± SEM) of 10 individual injections of cells. Each experiment was repeated in triplicate with essentially the same results.

**Generation and quenching of singlet molecular oxygen**

Singlet oxygen was generated chemically by thermo-decomposition of the endoperoxide of 3,3\(^\prime\)-(1,4-naphthylidene) dipropionate (NDPO\(_2\)) yielding \(^1\)O\(_2\) and \(^3\)O\(_2\) (16). At 37°C, 3 ml of de-aerated ethanol/chloroform (1/1) was placed into a thermostated, closed glass cuvette. The reaction was started by adding 50 \(\mu\)l of NDPO\(_2\) dissolved in D\(_2\)O to give a final concentration of 5 mM NDPO\(_2\). The infrared photoemission of \(^1\)O\(_2\) was monitored at 1270 nm using a liquid nitrogen-cooled germanium diode photodetector as described (16,17). At the maximum of the emission signal, which was achieved within 5–6 min, 50 \(\mu\)l of the carotenoid solution (in chloroform) were added. The overall quenching constant \((k_q + k_r)\) was calculated from Stern–Volmer plots, using the equation:

\[
S_o/S = 1 + (k_q + k_r) \tau [Q].
\]

\(S_o\) and \(S\) are the photoemission intensities in the absence and presence of the carotenoid, respectively; \(k_q\) is the physical quenching rate constant; \(k_r\) the chemical reaction rate; \(\tau\) the life time of \(^1\)O\(_2\) in the solvent; and \([Q]\) the concentration of the carotenoid.

**Results**

The structures of the different carotenoids are given in Figure 1. Apart from the naturally occurring carotenoids β-carotene, echinene, canthaxanthin, 4-hydroxy-β-carotene, cryptoxanthin (3-hydroxy-β-carotene), capsorubin and violerythrin, and the synthetic compounds retro-dehydro-β-carotene, dinor-
Fig. 2. Induction of GJC by β-carotene (◇), canthaxanthin (●), 4-hydroxy-β-carotene (□), retrodehydro-β-carotene (▲), C-30-dialdehyde (△) and tetrahydrofuran (control) (■). Confluent cultures of C3H/10T1/2 cells were treated every 5 days. GJC was measured with the dye-transfer assay as described in Materials and methods.

Fig. 3. Scatter plot for GJC and singlet oxygen quenching rate constants of different carotenoids. β-Carotene (◇), echinenone (○), canthaxanthin (●), 4-hydroxy-β-carotene (□), cryptoxanthin (3-hydroxy-β-carotene) (◆), retrodehydro-β-carotene (▲), capsorubin (◎), dinor-canthaxanthin (○), violerythrin (●), C-20-dialdehyde (▲), C-30-dialdehyde (△), C-40-dialdehyde (■). Data from Table I.

Discussion

The antioxidant properties of carotenoids and their influence on GJC have been discussed in context with their preventive effects towards the development of several kinds of cancer (18). However, little is known about properties of carotenoids, including synthetic analogs, other than β-carotene. With respect to the induction of GJC, we here identified a synthetic congener of β-carotene, retrodehydro-β-carotene, to be an efficient inducer of intercellular communication via gap junctions. This compound contains an additional double-bond in the hydrocarbon chain, and the ring systems are attached to the core by double-bonds, thus providing a co-planar carotenoid. It is interesting to note that even such major changes in the geometry of the molecule and its system of conjugated double-bonds have little influence on the biological activity. However, the data indicate that the presence of a six-membered ring is important for inducing GJC (Table I). None of the dialdehydes affected intercellular communication.

The carotenoids substituted with a five-membered ring showed only little activity in increasing GJC. A direct comparison between five- and six-membered substituents can be taken from dinor-canthaxanthin and canthaxanthin. The induction observed with the five-membered ring compound dimor-canthaxanthin is one-half that observed with the six-membered ring compound canthaxanthin. Thus, it appears likely that the size of the ring system of carotenoids plays a role with respect to biological effects on intercellular communication.

However, the position of oxygen atoms as substituent at canthaxanthin is lower than that of this dialdehyde, but canthaxanthin efficiently increases GJC.
confirm that non-provitamin A carotenoids also are efficient in inducing GJC and might be of importance in in vivo systems. Synthetic carotenoid derivatives with structures very different from the parent β-carotene are biologically active.

In contrast to the effects on GJC, the ring-system has only little influence on the singlet oxygen quenching properties of these compounds. Among the most effective quenchers of singlet oxygen are the unsubstituted C-30- and C-40-dialdehydes as well as the five-membered ring-compounds dinorcanthaxanthin and violerythrin. The quenching activities depend almost exclusively on the extent of the system of conjugated double bonds (19). The quenching rate constant increases in the series C-20-, C-30- and C-40-dialdehyde. Furthermore, retrodehydro-β-carotene is more efficient than β-carotene itself.

The scatter plot (Figure 3) showing little correlation between the effect of carotenoids on GJC and their singlet oxygen quenching properties is in line with similar previous results comparing effects of carotenoids on GJC with their inhibitory effects on lipid peroxidation (5). There is also no correlation between GJC reported here and the ranking of Trolox equivalent antioxidant capacity of carotenoids reported recently (20) (see Table I). Thus, the data indicate that antioxidant properties of carotenoids and effects on intercellular communication that are discussed as biochemical mechanisms underlying cancer-preventing potential may operate independently of each other.

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References


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