**β-Carotene breakdown products enhance genotoxic effects of oxidative stress in primary rat hepatocytes**

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Since it has to be expected that individuals exposed to oxidative stress who take supplements of β-carotene are simultaneously exposed to both β-carotene cleavage products (CPs) and oxidative stress, and both exposures have been demonstrated to cause genotoxic effects in primary rat hepatocytes, cytotoxic and genotoxic effects on primary rat hepatocytes after supplementation of the medium with increasing concentrations of a CP mixture during exposure to oxidative stress by treatment with either DMNQ (2,3-dimethoxy-1,4-naphthoquinone) or hypoxia/reoxygenation (Hy/Reox) was investigated. The cytological endpoints analysed were the mitotic indices, the percentages of apoptotic and necrotic cells, the percentages of micronucleated (MN) cells and the number of chromosomal aberrations (CAs) and sister chromatid exchanges (SCE). The results obtained clearly demonstrate that the CP mixture enhances the genotoxic effects of oxidative stress exposure, whereas it had no effect at all on the endpoints of cytotoxicity studied. These results further support the hypothesis that CP might be responsible for the reported carcinogenic response in the beta-Carotene and Retinol Efficacy Trial (CARET) and Alpha-Tocopherol Beta-carotene Cancer prevention (ATBC) chemoprevention trials.

Introduction

In a previous investigation we demonstrated that treatment of primary rat hepatocyte cultures with β-carotene did not induce any cytotoxic or genotoxic effects. On the other hand, exposure to a mixture of β-carotene breakdown products (CP) and one of the major β-carotene breakdown products, apo8'-carotenal, induced genotoxic effects (1). Notably, eccentric β-carotene cleavage products (CPs) are reported to be responsible for inducing cytotoxicity in primary hepatocytes (10). This might be expected under conditions of oxidative stress in the lungs of cigarette smokers (3) owing to the lowering of ascorbate and α-tocopherol, which play a stabilizing role on β-carotene (4,5). In this context, it appears noteworthy that smoking-induced oxidative stress causes CP formation at concentrations of 2–6 μM β-carotene, which represent physiologically relevant concentrations (6). Therefore, since β-carotene exposed to oxidative stress undergoes cleavage (6–8), it has to be expected that smokers who take supplements of β-carotene are exposed to both CP and oxidative stress simultaneously.

Owing to this observation and the existing evidence that CP are responsible for an increased risk of lung cancer in smokers and workers exposed to increased environmental pollution (2,3,6–8), potential modifications of the dose response to a CP mixture under conditions of oxidative stress were investigated, since this combination more likely reflects the actual situation. The CP mixture used was obtained upon degradation of β-carotene by hypochlorite and contains 5,6-epoxi-β-ionone, ionene, β-cyclodetrin, β-ionone, dihydroactinidiolide and 4-oxo-β-ionone, as detected by gas chromatography/mass spectrometry. From their chemical nature, these compounds have to be expected to be highly reactive (7,8) and, as previously reported, also proved to be genotoxic (1). Oxidative stress was induced either by the free-radical-generating compound DMNQ (2,3-dimethoxy-1,4-naphthoquinone) or by hypoxia/reoxygenation (Hy/Reox). DMNQ as a source of free radicals (9,10) and Hy/Reox have been demonstrated in primary hepatocytes to induce genotoxic effects (10). This investigation therefore focussed on possible modifications of these effects by CPs.

As a measure of genotoxicity, the percentages of micronucleated (MN) cells and the number of chromosomal aberrations (CAs) and sister chromatid exchanges (SCE) were analysed. Since the treatment regimens might also result in cytotoxicity, potential changes of the mitotic indices and the induction of apoptotic and necrotic cell death were further analysed.

Material and methods

**Materials**

Minimum essential medium (MEM) with Earle’s salts and non-essential amino acids, and antibiotics were obtained from Life Technologies, Vienna, Austria. Plastic culture dishes were purchased from Sarstedt, Austria. EGF, collagenase and other cell culture chemicals—unless otherwise specified—were purchased from Sigma Chemical Company via Biotrade, Vienna.

The generation of β-carotene breakdown products (CP) was performed as described by Siems et al. (7) and Sommerburg et al. (8) by C.-D. Langhans. For degradation, β-carotene was dissolved in methanol containing 2% v/v trichloromethane to achieve sufficient solubility of the carotenoid. Chemical destruction of β-carotene was done by bleaching with hypochlorite (adding NaOCl in a 100-fold concentration related to the carotenoid). The samples reacted at room temperature and daylight for 10 min. After hexane extraction different CP were identified (HPLC and GCMS) and partially quantified (HPLC) in the aliquots obtained. The CP mixture obtained from a 0.5 mM β-carotene stock solution contained β-carotene (0.16 mM), apo15'-carotenal (0.08 mM), apo12'-carotenal (0.12 mM), apo8'-carotenal (0.006 mM) and a...
number of products that could not be identified by HPLC. Further products could be identified by GCMS but not quantified because of the extraordinary technical difficulty. Related to all peaks detected during GCMS analysis, a peak area of roughly 4.8% accounted for \( \beta \)-cyclotrichlor. 0.1% for ionone, 9.9% for \( \beta \)-ionone, 1.9% for \( \beta \)-ionone-5,6-epoxide and 4.5% for dihydroacetinolide. Furthermore, 4-oxo-\( \beta \)-ionone was detected in traces.

**Animals.** Adult female Fischer 344 rats (8–14 weeks old) weighing \( \approx 100 \) g were obtained from Harlan–Winkelmann, Germany. The animals were kept in a temperature- and humidity-controlled room with a 12 h light–dark cycle. Food and water was provided *ad libitum*. Prior to hepatocyte isolation, the animals were allowed to acclimate for at least 2 weeks.

**Methods**

**Hepatocyte isolation and culture.** Hepatocytes were isolated by the two-step in situ collagenase liver perfusion technique as described previously (11). According to Eckl et al. (12,13) the isolated hepatocytes were plated at a density of 20,000 viable cells/cm² (5 \( \times \) 10⁶ cells/dish) on collagen-coated 60-mm diameter plastic culture dishes in 5 ml of serum-free MEM containing 1.8 mM calcium supplemented with non-essential amino acids, pyruvate (1 mM), aspartate (0.2 mM), serine (0.2 mM) and penicillin (100 U)/streptomycin (100 \( \mu \)g/ml). The cultures were incubated at standard culture conditions (37°C, 5% CO₂ and 95% relative humidity). After an initial incubation period of 3 h, the medium was exchanged for fresh MEM and the cultures were returned to the incubator.

**Treatment with DMNQ.** Forty-eight hours after the first exchange of the medium, DMNQ (Eubio, Vienna) at a concentration of 40 \( \mu \)M (made up fresh for each experiment) was added to the cultures alone or in combination with CPs at concentrations of 0.01, 0.1, 1 and 10 \( \mu \)M, and the cells were incubated for a further 3 h. Thereafter, the plates were washed twice with fresh medium to completely remove the applied substances and proliferative stimulation was carried out by adding fresh MEM (0.4 mM Ca²⁺) supplemented with insulin (0.1 \( \mu \)M) and EGF (40 \( \mu \)g/ml). To determine SCE induction, BrdU (10 \( \mu \)M) was added to three dishes of each concentration. Thereafter, the cultures were incubated for an additional 48 h.

**Hypoxia/reoxygenation.** Twenty-four hours after the first exchange of the medium, the cultures were transferred to a Hy/Reox chamber (Billups-Rothenberg, CA, USA) and exposed to hypoxic conditions (95% N₂ and 5% CO₂) for 24 h. After this, reoxygenation was carried out by changing the gas mixture to normoxic conditions (70% N₂, 25% O₂ and 5% CO₂) for a further 3 h. During reoxygenation, the cultures were treated with the CP mixture at concentrations of 0.01, 0.1, 1 and 10 \( \mu \)M. The experimental apparatus for Hy/Reox was placed in a warming chamber maintained at 37°C and the gas mixtures were bubbled continuously through sterile distilled water which was equilibrated to hypoxic or normoxic conditions 15 min prior to use. After 3 h of reoxygenation, proliferative stimulation and further steps were performed as described above.

**Fixation, staining and cytogenetic analysis.** Cytogenetic studies were performed as described by Eckl et al. (12,13). In brief, 48 h after proliferative stimulation, colcemide (0.4 \( \mu \)g/ml) was added to three dishes (where BrdU was added) per concentration, and the cultures were incubated for a further 3 h. No colcemide was added to the cultures used for the micronucleus assay. For chromosome preparations cells were harvested by collagenase (0.5 mg collagenase/ml) treatment for 10 minutes. The detached cells were collected by centrifugation (44 \( \times \)g), treated with hypotonic KCl solution (0.02M) for 10 min at 37°C and fixed in freshly prepared methanol : glacial acetic acid (3 : 1) overnight. Preparations were made by dropping the cell suspension on pre-cleaned frosted slides.

For the micronucleus assay, cells were fixed in the dishes with methanol : glacial acetic acid (3 : 1) for 15 min, briefly rinsed with distilled water and air dried. Thereafter, the fixed cells were stained with the fluorescent dye DAPI (4',6-diamidino-2-phenylindol) in McIlvaine buffer (0.2 M Na₂HPO₄ buffer adjusted to pH 7.0 with 0.1 M citric acid) for 30 min in the dark at room temperature. After washing with McIlvaine buffer, the dishes were rinsed with distilled water, air dried and mounted in glycerol.

Thousand cells per dish were analysed under the fluorescence microscope (Leitz Aristoplan) to determine the mitotic indices, the frequencies of apoptotic and necrotic cells and the number of cells with micronuclei. The morphology of DAPI-stained nuclei was used to discriminate between mitotic, apoptotic and necrotic cells as described previously (14).

The slides for studying CAs and SCE induction were stained with Hoechst 33258 (4.5μg/ml) in Sörensen phosphate buffer pH 6.8 for 20 min, rinsed with Sörensen phosphate buffer and exposed to black light (General Electric, F 40 BLB Black light) for 15 min on a warming plate kept at 50°C. After removal of the coverslips, the slides were rinsed briefly with distilled water and stained in 5% Giemsa solution. Twenty well-spread-first division-metaphases were analysed for SCE. The number of aberrations is given per diploid cell, that is, 42 chromosomes. The reported SCEs are calculated as SCEs per chromosome.

**Statistical analysis**

Student’s double-sided \( t \)-test for independent samples was used to calculate the levels of significance by use of the SPSS statistical software package.

**Results**

**Effects of CP under DMNQ-induced oxidative stress**

DMNQ applied alone at a concentration of 40 \( \mu \)M did not induce any cytotoxic effects; however, the percentages of MN cells, CAs and SCE were significantly (\( P < 0.05 \)) elevated (Table I).

Up to a concentration of 1 \( \mu \)M CPs applied in combination with 40 \( \mu \)M DMNQ (representing the lowest concentration of DMNQ that proved to be genotoxic with respect to all genotoxic endpoints analysed), no cytotoxic effects were detectable. Combined treatment of primary hepatocyte cultures with 10 \( \mu \)M CP and 40 \( \mu \)M DMNQ, however, was toxic as shown in Table II.

As demonstrated in Figure 1, the genotoxicity of DMNQ (when applied alone) was further increased by combined treatment with CPs applied at concentrations between 0.01 and 1 \( \mu \)M. The frequency of MN cells was further increased, the increases being significant (\( P < 0.05 \)) at 0.01 and 1 \( \mu \)M CP. In comparison, CAs induced by 40 \( \mu \)M DMNQ were not altered by simultaneous treatment with CPs at concentrations of 0.01 and 1 \( \mu \)M, but became significant (\( P < 0.05 \)) at 1 \( \mu \)M. Similarly, SCE induction also proved to be dose-dependent and was significantly (\( P < 0.05 \)) increased upon addition of 1 \( \mu \)M CP compared with treatment with DMNQ alone.

**Effects of CP under oxidative stress induced by Hy/Reox**

As shown in Table III Hy/Reox caused no cytotoxic effects by itself but induced high levels of genotoxicity. The percentage of MN cells was significantly higher (\( P < 0.05 \)) compared with that found in normoxic control cultures, which was also the case for CA (\( P < 0.005 \)) and SCE induction (\( P < 0.05 \)).

Addition of CP at concentrations of 0.01, 0.1, 1 and 10 \( \mu \)M during the reoxygenation phase of Hy/Reox did not cause any

**Table I. Cyto- and genotoxic effects of oxidative stress induced by DMNQ (40 \( \mu \)M) in primary rat hepatocyte cultures**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mitotic index (%)</th>
<th>Necrotic cells (%)</th>
<th>Apoptotic cells (%)</th>
<th>Micronucleated cells (%)</th>
<th>CA/metaphase</th>
<th>SCE/chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% DMSO)</td>
<td>1.00 ± 0.10</td>
<td>0.17 ± 0.15</td>
<td>0.23 ± 0.12</td>
<td>7.37 ± 0.59</td>
<td>0.10 ± 0.01</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td>DMNQ (40μM) in 1% DMSO</td>
<td>1.00 ± 0.26</td>
<td>0.23 ± 0.12</td>
<td>0.27 ± 0.12</td>
<td>8.60 ± 0.53</td>
<td>0.20 ± 0.02</td>
<td>0.63 ± 0.07</td>
</tr>
</tbody>
</table>

*Represents the highest concentration of DMSO applied. Data represent the means ± SD of three independent experiments. \( P \leq 0.05 \) compared with the control.
cytotoxic effect (Table IV), but gave rise to prominent genotoxic effects as demonstrated in Figure 2.

Under this regimen, the frequency of MN cells was significantly increased upon the addition of CPs at concentrations of 0.1 μM ($P < 0.005$), 1 μM ($P < 0.05$) and 10 μM ($P < 0.005$) compared with Hy/Reox alone. This treatment also led to a dose-dependent increase of CAs, which became significant at concentrations $\geq 0.1$ μM: 0.1 μM ($P < 0.05$), 1 μM ($P < 0.005$) and 10 μM ($P < 0.005$). The dose response of SCE induction showed a comparable manner and became significant ($P < 0.05$) at 1 and 10 μM.

Interestingly, the combined treatment with Hy/Reox and CP led to the appearance of metaphases with a high number of aberrations at all CP concentrations tested. In particular, at concentrations of 1 μM and 10 μM CP, highly aberrant metaphases showing 10 or more aberrations per metaphase appeared at a frequency of 1/30 of the analysed metaphases. An example for such a metaphase is given in Figure 3 showing gaps, chromosomal fragments, paired fragments, ring chromosomes as well as acentric, dicentric and tricentric chromosomes. It should be noted here that these highly aberrant metaphases have been excluded from the statistical analysis of CAs.

**Discussion**

In this study we demonstrate that the genotoxic potential of experimentally induced oxidative stress is significantly enhanced by β-carotene CPs. Cleavage of β-carotene by oxidative stress (6–8,15,16) gives rise to the formation of high amounts of β-carotene breakdown products (CPs) with prooxidant (7,8) and genotoxic (1) properties. Furthermore, it has

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**Table II.** Effects of CP on cytotoxicity endpoints under DMNQ-induced oxidative stress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mitotic index (%)</th>
<th>Necrotic cells (%)</th>
<th>Apoptotic cells (%)</th>
<th>Micronucleated cells (%)</th>
<th>CA/metaphase</th>
<th>SCE/chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (40 μM DMNQ/1% DMSO)</td>
<td>1.07 ± 0.06</td>
<td>0.10 ± 0.10</td>
<td>0.27 ± 0.06</td>
<td>0.58 ± 0.05</td>
<td>0.08 ± 0.01</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>DMNQ + 0.01 μM CP</td>
<td>1.10 ± 0.10</td>
<td>0.27 ± 0.23</td>
<td>0.48 ± 0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMNQ + 0.1 μM CP</td>
<td>1.00 ± 0.10</td>
<td>0.17 ± 0.19</td>
<td>0.23 ± 0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMNQ + 1 μM CP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMNQ + 10 μM CP</td>
<td>Toxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Represents the highest concentration of DMSO applied. Data represent the means ± SD of three independent experiments.

**Fig. I.** Frequencies of MN cells (A), CAs (B) and SCE (C) in cultures treated with 40 μM DMNQ (open squares) and cultures simultaneously treated with DMNQ + CP (closed squares). Data represent the means ± SEM of three independent experiments. *$P \leq 0.05$.

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**Table III.** Cyto- and genotoxic effects of Hy/Reox-induced oxidative stress on primary rat hepatocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mitotic index (%)</th>
<th>Necrotic cells (%)</th>
<th>Apoptotic cells (%)</th>
<th>Micronucleated cells (%)</th>
<th>CA/metaphase</th>
<th>SCE/chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% DMSO$^*$)</td>
<td>0.91 ± 0.31</td>
<td>0.10 ± 0.05</td>
<td>0.95 ± 0.37</td>
<td>3.96 ± 0.65</td>
<td>0.08 ± 0.01</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>Hy/Reox (1% DMSO$^*$)</td>
<td>1.37 ± 0.60</td>
<td>0.06 ± 0.03</td>
<td>0.20 ± 0.09</td>
<td>6.86 ± 0.45**</td>
<td>0.31 ± 0.02**</td>
<td>0.58 ± 0.05*</td>
</tr>
</tbody>
</table>

$^*$Represents the concentration of DMSO at the highest concentrations applied. Data represent the means ± SD of three independent experiments.

* $P \leq 0.05$; ** $P \leq 0.005$ compared with the control.
been suggested that CPs contribute to carcinogenic events in the lungs of cigarette smokers (2,3,17,18). This indicates their responsibility for adverse side effects of β-carotene supplementation reported in the Alpha-Tocopherol, Beta-carotene Cancer-Prevention (ATBC) study and the Beta-Carotene and Retinol Efficacy Trial (CARET) (19,20). In particular, it has been reported that the chemical structure of many of these CPs (i.e. epoxides) is unstable under conditions of oxidative stress, and thus CPs themselves may reveal pro-oxidant properties (21). In addition, inflammatory processes in lungs exposed to increased oxidative stress—as it is the case in smokers and asbestos workers—may lead to the formation of carotenoid oxidation products (21). Importantly, evidence for β-carotene-mediated genotoxicity under conditions of oxidative stress is provided by the observation that CAs are increased in Chinese hamster ovary cells (22) simultaneously exposed to β-carotene and H2O2. The same treatment has also been demonstrated to promote DNA strand breaks in human HepG2 hepatoma cells (23).

In a previous investigation CPs were demonstrated to be genotoxic in primary rat hepatocytes (1); however, it remained to be clarified how this effect is modified under oxidative stress, since it can be assumed that both CPs and oxidative stress contribute to lung damage during smoking. The results presented demonstrate that oxidative stress induced by DMNQ, a compound that is considered to cause DNA single-strand breaks via the formation of OH-radicals (24), exerts genotoxic effects in primary hepatocytes, which is in good accordance with other reports on DMNQ-mediated genotoxicity (10). CPs applied at concentrations between 0.01 and 1 μM further enhance the genotoxicity induced by DMNQ in a dose-dependent manner as evidenced by a further shift of the numbers of MN cells and an increased incidence of CAs and SCEs. Compared with the previous results on CP genotoxicity (1) the combined treatment with CPs and DMNQ lead to significantly higher percentages of MN cells at 0.01 and 1 μM (P < 0.05), significantly higher numbers of CAs at 0.01 μM (P < 0.01) and significantly higher numbers of SCE at 0.01, 0.1 and 1 μM (P < 0.01). The genotoxic effects of Hy/Reox were also enhanced by simultaneous treatment with CPs during the reoxygenation phase. Several effects have been described for Hy/Reox, among which the generation of free radicals most probably is responsible for its genotoxic potential (10,25–27). Compared with the previous results the combined treatment with CPs and Hy/Reox did not lead to significant changes of micronucleus induction eventually owing to the high variations between the experiments, whereas there was a significant effect on CAs and SCE: the numbers of CAs were significantly increased at 0.01 (P < 0.05), 1 and 10 μM (P < 0.01); SCE were significantly elevated (P < 0.01) at all concentrations tested. Therefore, in extension to the previous results, this study demonstrates that CPs enhance the genotoxic effects of oxidative stress in primary hepatocytes. As far as the underlying mechanisms are concerned, CPs have been shown to exert their effects by inducing cytochrome P450 enzyme activity, to decrease retinoic acid

![Fig. 2](https://academic.oup.com/carcin/article-abstract/27/6/1128/2391008)

**Table IV.** Effects of CP on cytotoxicity endpoints under Hy/Reox-induced oxidative stress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mitotic index (%)</th>
<th>Necrotic cells (%)</th>
<th>Apoptotic cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Hy/Reox/ 1% DMSO)</td>
<td>1.37 ± 0.60</td>
<td>0.06 ± 0.03</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>Hy/Reox + 0.01 μM CP</td>
<td>1.53 ± 0.26</td>
<td>0.03 ± 0.02</td>
<td>0.10 ± 0.09</td>
</tr>
<tr>
<td>Hy/Reox + 0.1 μM CP</td>
<td>1.41 ± 0.24</td>
<td>0.03 ± 0.03</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td>Hy/Reox + 1 μM CP</td>
<td>1.51 ± 0.30</td>
<td>0.08 ± 0.06</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Hy/Reox + 10 μM CP</td>
<td>1.35 ± 0.60</td>
<td>0.03 ± 0.03</td>
<td>0.10 ± 0.10</td>
</tr>
</tbody>
</table>

aRepresents the highest concentration of DMSO applied. Data represent the means ± SD of three independent experiments.

**Fig. 2.** Frequencies of MN cells (A), CAs (B) and SCE (C) in control cultures (Hy/Reox; open squares) and cultures treated with different concentrations of CP during the reoxygenation phase (closed squares). Data represent the means ± SEM of three independent experiments. *P ≤ 0.05; **P ≤ 0.005.
levels and to diminish retinoid signalling leading to lung cell proliferation (3). Especially apo-8-carotene has been reported to be a strong inducer of liver cytochromes P450 1A1 and 1A2 (28). The same compound has been shown to be genotoxic in primary rat hepatocytes inducing MN cells, CAs and SCE (1). Furthermore, owing to the described pro-oxidant properties of CPs (7,8) it can be assumed that these compounds also enhance lipid peroxidation initiated by DMNQ and Hy/Reox (29) eventually resulting in the formation of aldehydic lipid peroxidation products, among which 4-hydroxynonenal (HNE) has been shown to be a potent inducer of cytotoxic and genotoxic effects (30).

Chromosomal instability has been used extensively as a biomarker for an early effect of genotoxic carcinogens (31), and the fact that genotoxic carotenogens induce CAs and cancer highlights the increased incidence of CAs as an intermediate endpoint of carcinogenesis (32). Therefore, the appearance of highly aberrant metaphases in preparations from cultures exposed to Hy/Reox and CPs is of special importance: Usually a decrease of the mitotic index with increasing levels of CAs or other endpoints of genotoxicity is observed, that is, in hepatocytes (13). In parallel, increasing numbers of necrotic and/or apoptotic cells are reported (10), indicating that (heavily) damaged cells undergo active or passive cell death. The lack of cytotoxic effects accompanied by the appearance of metaphases with large numbers of CAs therefore most probably has to be interpreted as an inhibition of apoptosis. To test this assumption the role of apoptosis regulating gene products such as (i) proapoptotic p53, FasL, and Bid; (ii) apoptosis suppressing bcl-xL; and (iii) the impairment of mitochondria (cytochrome c release) will be studied parallel to the morphological quantification of apoptosis rates.

Finally, it has to be noted that the oxidizing environment of cigarette smoke decreases the levels of antioxidants such as ascorbate and α-tocopherol, which stabilize the oxidized β-carotene (3). Therefore, the lack of β-carotene stabilizing antioxidants may promote the formation of CPs in the free-radical-rich environment in the lungs of cigarette smokers, which as a consequence may contribute to the carcinogenic response (33). Importantly, individuals participating in the ATBC study and CARET were individuals exposed to oxidative stress, that is, smokers and asbestos workers. Receiving supplementary β-carotene they can be expected to be exposed to both CPs and oxidative stress at the same time. The finding that CPs enhance the genotoxic effects of oxidative stress therefore can further contribute to the understanding of the adverse effects found in cancer prevention studies.

Conflict of Interest Statement: None declared.

References


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