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Original Contribution

β-Carotene metabolites enhance inflammation-induced oxidative DNA damage in lung epithelial cells

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ABSTRACT

β-Carotene (BC) intake has been shown to enhance lung cancer risk in smokers and asbestos-exposed subjects (according to the ATBC and CARET studies), but the mechanism behind this procarcinogenic effect of BC is unclear. Both smoking and asbestos exposure induce an influx of inflammatory neutrophils into the airways, which results in an increased production of reactive oxygen species and formation of promutagenic DNA lesions. Therefore, the aim of our study was to investigate the effects of BC and its metabolites (BCM) on neutrophil-induced genotoxicity. We observed that the BCM vitamin A (Vit A) and retinoic acid (RA) inhibited the H_2O_2-utilizing enzyme myeloperoxidase (MPO), which is released by neutrophils, thereby reducing H_2O_2 conversion. Moreover, BC and BCM were able to increase $^{\cdot}OH$ formation from H_2O_2 in the Fenton reaction (determined by electron spin resonance spectroscopy). Addition of Vit A and RA to lung epithelial cells that were co-incubated with activated neutrophils resulted in a significant increase in the level of oxidized purines assessed by the formamidopyrimidine DNA glycosylase-modified comet assay. These data indicate that BCM can enhance neutrophil-induced genotoxicity by inhibition of MPO in combination with subsequent increased formation of hydroxyl radicals.

β-Carotene (BC) is a naturally occurring orange-colored phytochemical widely used as a coloring agent in food products and added to vitamin supplements. BC is considered to be an effective antioxidant and thought to decrease cancer risk, especially lung cancer [1,2]. Two large-scale intervention trials, the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC study) and the Carotene and Retinol Efficacy Trial (CARET study), investigated the effect of BC supplementation alone (ATBC) or in combination with vitamin A (CARET) in subjects at risk for lung cancer owing to smoking or asbestos exposure. The effect of α-tocopherol, the predominant form of vitamin E (Vit E), was also explored in the ATBC study. Unexpectedly, BC supplementation increased lung cancer incidence in both the ATBC and the CARET study, whereas Vit E supplementation did not show any effect [3,4]. The mechanism leading to this increased lung cancer risk is not known and remains to be elucidated.

Different mechanisms are involved in the carcinogenicity of asbestos and smoking, but both exposures cause an inflammatory response in the lungs and are associated with the onset and progression of carcinogenesis [5–9]. A role for chronic inflammation in lung carcinogenesis is demonstrated by studies in which the use of anti-inflammatory drugs for at least 1 year resulted in a reduction in the relative risk of lung cancer in smokers [10,11]. Typical of lung inflammation is the influx of neutrophils into the airways [12,13].

Activated neutrophils display a respiratory burst, generating reactive oxygen species (ROS); superoxide anions ($O_2^{\cdot-}$) are formed, which can be converted into hydrogen peroxide (H_2O_2) [13]. Subsequently, H_2O_2 can either be converted into hypochloric acid (HOCl) by myeloperoxidase (MPO), an enzyme released by activated neutrophils, or react with transition metals to form highly reactive

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hydroxyl radicals (·OH) via the Fenton reaction. Both HOCl and ·OH are highly reactive and are able to peroxidize proteins and lipids and to react with DNA. However, owing to their high reactivity it is unlikely that they diffuse into the nucleus when generated extracellularly [14–16]. In contrast, H_2O_2 is relatively stable and able to diffuse into the nucleus, where it can react with DNA-associated transition metals to form ·OH, causing a variety of DNA lesions, including single-strand DNA breaks (ssDNA) and oxidized DNA bases [17]. Moreover, H_2O_2 probably has a greater genotoxic potential than HOCl because inhibition of MPO led to an increase in ssDNA breaks in RLE lung epithelial cells co-incubated with neutrophils [18]. Oxidatively damaged DNA is known to be promutagenic and is therefore an important marker for carcinogenesis. In this respect, catalase is considered an important antioxidant enzyme in alveolar cells because it converts H_2O_2 into water and oxygen, thereby preventing H_2O_2-induced damage [19].

High BC intake was associated with increased lung cancer risk in smokers and asbestos-exposed subjects. Both asbestos exposure and smoking cause a neutrophilic influx, which is suggested to be a mediating factor in lung cancer development [13]. To examine a possible role for BC in neutrophil-mediated carcinogenesis, we investigated the effects of BC on neutrophil-induced oxidative DNA damage in A549 type II lung epithelial cells. The effects of BC on the generation of O_2^- and ·OH were tested in vitro using electron spin resonance (ESR) spectroscopy in combination with the spin trapping technique. Subsequently, the effects of BC on MPO and catalase activity were measured. As BC can be metabolized in the human body [20], the BC metabolites (BCM) retinoic acid (RA), retinal (RAL), and vitamin A (Vit A) were also included. Vit E was used to test the specificity of the observed effects because Vit E is a lipophilic vitamin, like BC, but had no observed effect on lung cancer incidence in the ATBC study.

Materials and methods

Preparation of vitamin solutions

To study the effects of BC supplementation on lung carcinogenesis in smokers, vitamin concentrations were based on serum concentrations that were observed in the CARET and ATBC study after 3 years of daily vitamin intake [21]. Analogous to BC serum concentrations in these studies, 5 μM BC was used in our in vitro experiments. Corresponding to this BC concentration, 10 μM BCM were used in our in vitro experiments because BC can be metabolized and cleaved into two molecules, RAL, which can subsequently be metabolized into Vit A, or RA. Based on Vit E serum concentrations after 3 years of daily Vit E intake (ATBC) [21], 40 μM Vit E was used in the in vitro experiments. Stock solutions of all-*trans*-β-carotene (15 mM), retinol (30 mM), retinal (30 mM), retinoic acid (30 mM), and α-tocopherol (120 mM) (all from Sigma–Aldrich) were prepared in deperoxidized tetrahydrofuran (THF) and stored in amber vials under argon at −80 °C until use. THF was deperoxidized over an ALOX column (aluminum oxide-90 active basic; Merck). Stock solutions were diluted 1:30 in heat-inactivated fetal calf serum (FCS; Gibco) to make the vitamins more soluble in hydrophilic solutions. Solvent containing 0.033% THF and 1% FCS was used as control (Co).

Radical measurement by ESR spectroscopy

Phenazine methosulfate (PMS), β-nicotinamide adenine dinucleotide (NADH; reduced form), iron sulfate ($FeSO_4$), H_2O_2, and the spin trap 5,5-dimethyl-1-pyrolline *N*-oxide (DMPO) were purchased from Sigma–Aldrich. DMPO was dissolved in nitrogen-flushed water and purified by charcoal treatment. Concentrations of DMPO stocks were determined spectrophotometrically (ε=7700 M^{-1} cm^{-1}, 234 nm). ESR spectra were recorded at room temperature in glass capillaries (100 μl; Brand AG) on a Bruker EMX 1273 spectrometer equipped with an ER 4119HS high-sensitivity cavity and 12-kW power supply operating at X band frequencies. The modulation frequency of the spectrometer was 100 kHz. Instrumental conditions for the recorded spectra were magnetic field 3490 G, scan range 60 G, modulation amplitude 1 G, receiver gain 1×10^5, microwave frequency 9.85 GHz, power 50 mW, time constant 40.96 ms, scan time 20.97 s, number of scans 10. Surface area and hyperfine coupling constants of the peaks were calculated using the WIN-EPR spectrum manipulation program (Bruker, Germany).

To assess ·OH formation, $FeSO_4$ (20 μM) was mixed with DMPO (100 mM) and solvent with or without BC, BCM, or Vit E. H_2O_2 (100 μM) was added to start the reaction. The measurement of O_2^- formation was accomplished by a reaction containing PMS (3.3 μM) mixed with DMPO (100 mM) and solvent with or without BC, BCM, or Vit E. Addition of NADH (50 μM) started the generation of O_2^-. In the ESR spectrum DMPO-trapped O_2^- (DMPO/·OOH) and DMPO-trapped ·OH (DMPO/·OH) signals arise. Wilms et al. previously demonstrated that the PMS/NADH system generates solely O_2^- because complete inhibition of the radical signals is established upon treatment by superoxide dismutase (SOD). The DMPO/·OH signal is generated because DMPO/·OOH decomposes into DMPO/·OH [22]. Exactly 2 min after the addition of H_2O_2 or NADH the spectrum was recorded. Experiments were performed in triplicate.

Neutrophil isolation

Polymorphonuclear neutrophils (PMN) consist of basophilic, eosinophilic, and neutrophilic granulocytes. Because the neutrophilic granulocytes are the most abundant fraction, we refer to PMN as neutrophils throughout this paper. The blood of healthy, nonsmoking, human donors was obtained by venapuncture and diluted 1:1 with Hanks' balanced salt solution (HBSS; Gibco). Lymphocytes were removed by gradient centrifugation and subsequently erythrocytes were lysed by adding 2 volumes of cold lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, and 10 mM EDTA, pH 7.4). Neutrophils were washed and resuspended in HBSS and counted and viability was determined by trypan blue exclusion. Neutrophil viability was always >95%. Solutions and neutrophils were kept on ice during the whole procedure.

MPO activity measurement

Neutrophils (1×10^7) were dissolved in 10 ml HBSS and incubated for 15 min at 37 °C in a 75-cm^2 culturing flask. Thereafter, phorbol 12-mystrate 13-acetate (PMA; Sigma–Aldrich) was added (100 ng/ml) and the incubation was continued for another hour at 37 °C. MPO activity in the supernatant was immediately determined in a 96-well plate based on the method described by Klebanoff et al. [23] with some modifications: 40 μl supernatant was mixed with 20 μl solvent, BC, BCM, or Vit E and 140 μl assay solution (containing 10 mM sodium phosphate buffer, 0.33 mM H_2O_2, and 11% guaiacol). The generation of tetraguaiacol is a measure of MPO activity and was assessed spectrophotometrically at 470 nm. MPO activity was calculated using the formula U/ml=ΔOD/min×1.203, as a percentage of control.

The whole procedure was also performed without the presence of neutrophils to examine the effects of solvent, BC, BCM, and Vit E on the formation of tetraguaiacol as such. No effect of the vitamins on the formation of tetraguaiacol was observed.

Coculture with neutrophils

The human epithelial lung carcinoma cell line A549 was cultured in DMEM (Sigma–Aldrich) supplemented with 10% heat-inactivated FCS (Life Technologies, Invitrogen) and 1% penicillin and 1% streptomycin (Sigma–Aldrich) at 37 °C in a 5% CO_2 atmosphere. Coculture experiments were performed as described by Knaapen et al. [24]. A549 cells were cultured in a six-well plate in medium containing

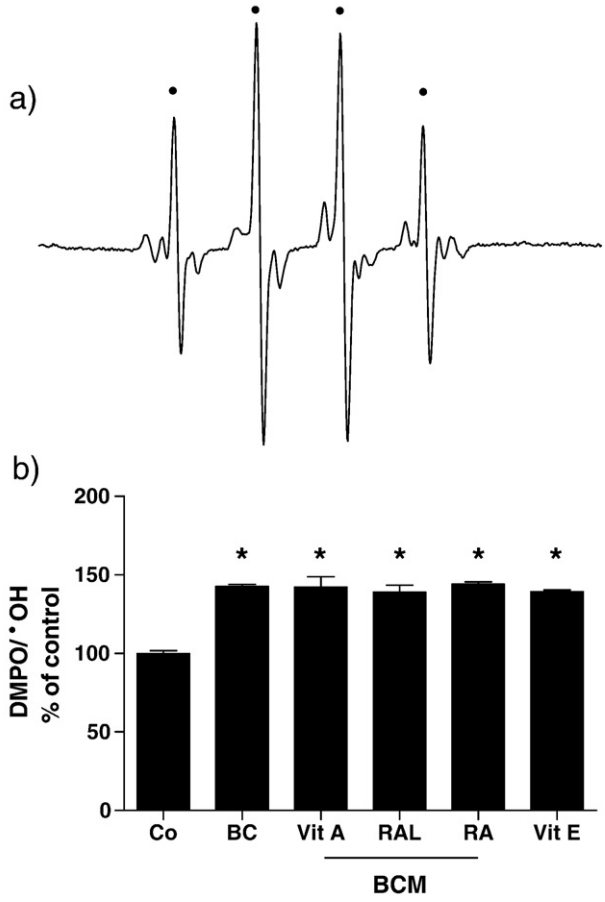

Fig. 1. (a) ESR spectrum of an Fe^{2+}/H_2O_2 reaction containing DMPO (100 mM), $FeSO_4$ (20 μM), and H_2O_2 (100 μM) in solvent. Dots indicate the DMPO/˙OH adduct signal. (b) Surface area of the DMPO/˙OH peaks in the absence (Co) or presence of BC (5 μM), BCM (10 μM), or Vit E (40 μM) was recorded and is expressed as % of control. BC (5 μM), BCM (10 μM), and Vit E (40 μM) increased the DMPO/˙OH radical formation significantly. *$p<0.001$; error bars indicate SEM.

solvent, BC, BCM, or Vit E, starting 24 h before the co-incubation. One confluent well contained approximately 1×10^6 cells. Cells were washed with HBSS and thereafter neutrophils (1×10^6 cells) in HBSS containing solvent (Co), BC, BCM, or Vit E were added to the wells in a total volume of 400 μl and incubated at 37 °C for 15 min. Thereafter PMA was added (100 ng/ml) and the cells were incubated for 1 h at 37 °C. After incubation the cells were washed with HBSS without calcium chloride, magnesium chloride, or magnesium sulfate to remove the neutrophils. Epithelial cells were harvested and resuspended in HBSS at a concentration of 2×10^6 cells/ml.

Formamidopyrimidine DNA glycosylase (FPG)-modified comet assay

Twenty-five microliters of cell suspension (2×10^6 cells/ml) was mixed with 75 μl low-melting-point agarose (0.65% in PBS). Seventy-five microliters of suspension was transferred to a coated (1.5% normal agarose in PBS) microscopic slide, covered with a coverslip, and stored at 4 °C to solidify the low-melting-point agarose. Slides were subsequently lysed (16 h) at 4 °C in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 250 mM NaOH, 10% DMSO, and 1% Triton X-100). After lysis, the slides were washed three times in 40 mM HEPES, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/ml BSA, pH 8.0. Slides were incubated for 10 min at 37 °C with 50 μl FPG (2.5 μg/ml, kindly provided by Bernd Epe, University of Mainz, Mainz, Germany) and subsequently placed on ice to stop the reaction. FPG specifically recognizes and cuts oxidized purines. Slides were incubated in 300 mM NaOH and 1 mM EDTA for 20 min to unwind and denature DNA. Thereafter electrophoresis was performed for 20 min at 300 mA and 25 V. Slides were rinsed three times in cold 0.4 M Tris solution and rinsed three times with 100% ethanol. Dry slides were stained with ethidium bromide (10 μg/ml) and 50 cells per slide were blindly scored using the comet assay III (Perceptive Instruments) program and the Δ (FPG treated − untreated) median tail moment was used for the level of oxidized purines in the DNA.

Catalase activity measurement

Catalase activity was measured spectrophotometrically in triplicate as described by Morikawa et al. [25]. Briefly, 100 μl assay buffer containing 3 mU/ml catalase and vitamins was mixed with 75 μl Purpald (Sigma–Aldrich) (25 mM in 2 M potassium hydroxide). The reaction was stopped after 2 min by adding 25 μl of potassium periodate (65.2 mM) and absorbance was recorded at 540 nm. Catalase activity was measured as the percentage of control. The whole procedure was also performed without catalase to examine the effects of solvent, BC, BCM, and Vit E on the oxidation of Purpald. No effect of the vitamins on the formation of the purple color was observed.

Statistics

Each measurement was performed at least in triplicate for each subject ($N=6$). Triplicates were averaged for each subject and results are presented as the mean ± SEM. Statistical analysis was performed in

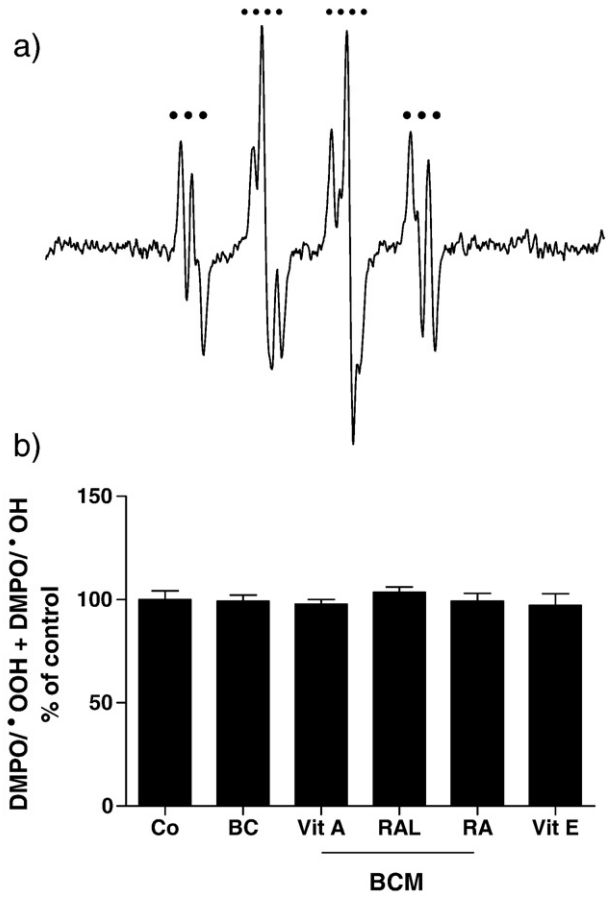

Fig. 2. (a) ESR spectrum of a PMS/NADH reaction containing DMPO (100 mM), PMS (3.3 μM), and NADH (50 μM) in solvent. Dots indicate the combined DMPO/˙OH and DMPO/˙OOH adduct signal. (b) Surface area of the DMPO/˙OH + DMPO/˙OOH peaks in the absence (Co) or presence of BC (5 μM), BCM (10 μM), or Vit E (40 μM) was recorded and is expressed as % of control. There were no differences in total DMPO/˙OH + DMPO/˙OOH radical formation between Co and BC, BCM, or Vit E. Error bars indicate SEM.

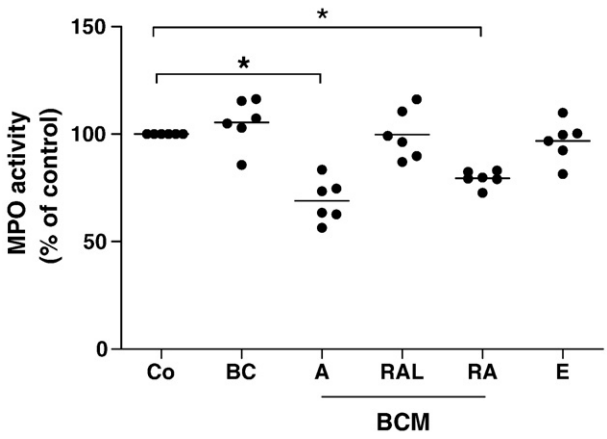

Fig. 3. MPO activity in the supernatant of PMA-activated neutrophils from six volunteers, measured in the absence (Co) or presence of BC (5 μM), BCM (10 μM), or Vit E (40 μM). Data are expressed as % of control. RA (10 μM) and Vit A (10 μM) decreased MPO activity significantly. *$p<0.05$; error bars indicate SEM.

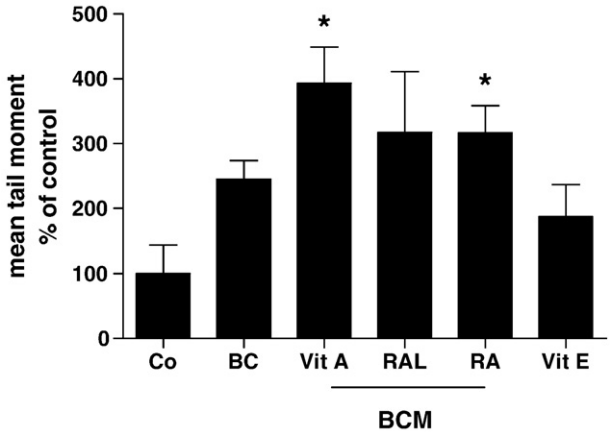

Fig. 5. Level of oxidized purines (% of control) in A549 epithelial cells after co-incubation with PMA-activated neutrophils in the absence (Co) or presence of BC (5 μM), BCM (10 μM), or Vit E (40 μM), as measured by the FPG-modified comet assay. Vit A and RA increased mean tail moment compared to the control. *$p<0.05$; error bars indicate SEM.

SPSS (version 15.0) for windows using ANOVA and subsequent Student *t* tests to compare incubation of BC, BCM, or Vit E versus Co. Trend analysis was performed using linear regression analysis on dose–response relationships. Differences were considered statistically significant at $p<0.05$.

Results

Effect of vitamins on ROS formation

During neutrophil-induced oxidative stress, $O_2^{\cdot-}$ is formed and converted into H_2O_2, which subsequently can react with transition metals to form $^{\cdot}OH$. The effects of BC, BCM, or Vit E on $O_2^{\cdot-}$ and $^{\cdot}OH$ formation was assessed by ESR spectroscopy [22].

The DMPO/$^{\cdot}OH$ signal was predominant in the Fe^{2+}/H_2O_2 system (coupling constants: a(N)=14.86 G, a(β-H)=14.71 G) (Fig. 1a). A significant increase of approximately 40% in $^{\cdot}OH$ formation ($p<0.001$) by BC, BCM, and Vit E was observed in the Fe^{2+}/H_2O_2 system (Fig. 1b).

The $O_2^{\cdot-}$ generated in the PMS/NADH system was measured as the sum of DMPO/$^{\cdot}OH$ and DMPO/$^{\cdot}OOH$ (coupling constants: a(N)=14.10 G, a(β-H)=11.05 G, and a(γ-H)=1.18 G for the DMPO/$^{\cdot}OOH$ signal) (Fig. 2a). Equal amounts of DMPO/$^{\cdot}OOH$+DMPO/$^{\cdot}OH$ were formed in the presence of BC, BCM, and Vit E compared to the control (Fig. 2b).

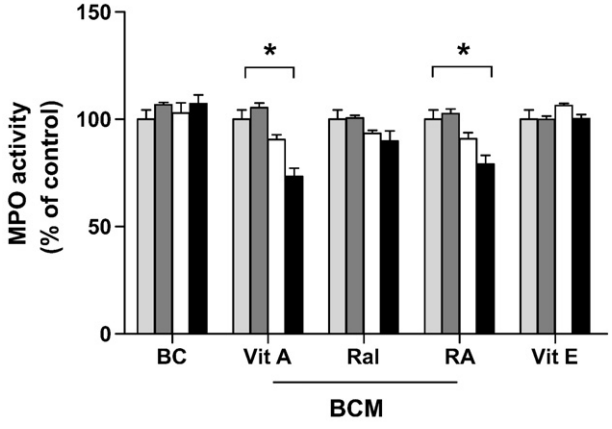

Fig. 4. MPO activity in the supernatant of PMA-activated neutrophils, measured in the absence (Co; 0%) (light gray bars) or presence of 4% (dark gray bars), 20% (white bars), or 100% (black bars) BC (5 μM), BCM (10 μM), or Vit E (40 μM) and expressed as % of control. There was a significant dose-dependent inhibition of MPO activity by RA and Vit A. *p for trend <0.001; error bars indicate SEM.

Effect of vitamins on H_2O_2-consuming enzymes: MPO and catalase

MPO activity was measured in the supernatant of PMA-activated neutrophils of six healthy nonsmoking human donors (two females, four males, ages 25–50 years). Vit A and RA effectively inhibited MPO activity by 31 and 21%, respectively (Fig. 3). BC, RAL, and Vit E did not affect MPO activity. There was a dose-dependent (p for trend <0.001) inhibition of MPO activity by Vit A and RA (Fig. 4). Catalase activity was not affected by BC, BCM, or Vit E.

Effect of vitamins on the level of oxidized purines in the DNA of lung epithelial cells induced by cocultured neutrophils

Supplementation with Vit A and RA resulted in a four- and threefold increase, respectively, in neutrophil-induced oxidative DNA damage, whereas there was no significant increase in the level of oxidized DNA when cells were exposed to BC, RAL, or Vit E (Fig. 5). The potency of the vitamins to enhance FPG-labile sites correlated significantly with their capacity to inhibit MPO activity ($r=-0.82$, $p<0.05$).

Discussion

We examined the effects of BC and BCM on neutrophil induced genotoxicity as a possible mechanism to explain the adverse health effects of BC supplementation that have been observed in smokers and asbestos-exposed subjects. Here, we demonstrate that the BCM RA and Vit A significantly increase the levels of oxidized purines in DNA of A549 lung epithelial cells that were co-incubated with neutrophils. We show that this increase is, at least in part, due to an increased formation of $^{\cdot}OH$ and decreased MPO activity. Our proposed mechanism is depicted in Fig. 6. Active MPO normally converts 40–70% of all neutrophil-generated H_2O_2 (Fig. 6, step 1) to generate HOCl (Fig. 6, step 2) [26]. We demonstrate that Vit A and RA are able to decrease MPO activity, thereby increasing the production of genotoxic $^{\cdot}OH$ radicals (Fig. 6, step 3) that form promutagenic DNA lesions.

A relation between MPO activity and lung cancer risk was studied before; the −436G→A polymorphism in the *MPO* promoter region was assessed in a number of epidemiological studies on lung cancer. The A allele, which is related to reduced MPO transcriptional activity [27] and lower MPO activity [28], was in some [29,30], but not all [31], studies associated with reduced rather than increased lung cancer risk. This seems to contradict our hypothesis. However, we stress that most of these studies focused on subjects with a normal diet without additional intake of BC. In fact, one of the studies that failed to

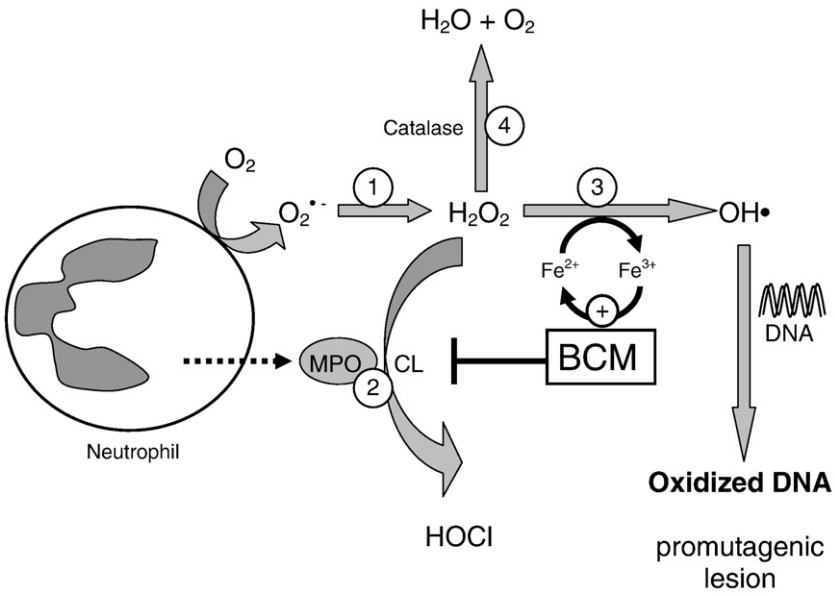

Fig. 6. Proposed mechanism of the effects of BCM on inflammation-induced oxidation of DNA in lung epithelial cells. During the respiratory burst $O_2^{\cdot-}$ is generated and converted into H_2O_2 (step 1). H_2O_2 can be converted to HOCl by MPO, which is released by activated neutrophils (step 2), or it can react with transition metals to form $^{\cdot}OH$ (step 3), which is able to oxidize DNA. The BCM Vit A and RA decrease MPO activity (step 2). This will yield higher local concentrations of H_2O_2 at the site of inflammation, which is subsequently converted to $^{\cdot}OH$ in the Fenton reaction (step 3). The Fenton reaction is more efficient in the presence of BCM, thereby increasing the level of $^{\cdot}OH$ and DNA oxidation. Alternatively, H_2O_2 can be converted into water and oxygen by catalase (step 4), but catalase activity was not affected by any of the vitamins.

demonstrate a reduced lung cancer risk for carriers of the A allele was nested within the ATBC study and included subjects with relatively high BC and Vit E levels due to the intervention [31]. The relatively high vitamin status in half of the subjects could counteract the protective effect of the A allele. Moreover, epidemiological studies are often unable to fully characterize the level of inflammation by actual measurements of MPO and factors that modulate its activity. For instance, the study by Van Schooten et al. [28] found large interindividual variations in MPO activity, even between subjects with the same *MPO* genotype and similar levels of exposure to cigarette smoke. Our study indicates that it is important to correct for possible confounding factors such as BC intake in studying the relationship between MPO and lung cancer risk in smokers and asbestos-exposed subjects.

Iron is an important constituent of smoke and asbestos, and iron concentrations are indeed elevated in lungs of smokers and subjects exposed to asbestos [32,33]. Intracellular concentrations of iron are important in neutrophil-induced DNA damage because most neutrophil-derived H_2O_2 is generated outside the activated neutrophils during the respiratory burst [34] and then diffuses into the nucleus and reacts with DNA-associated iron to form highly genotoxic hydroxyl radicals. In vitro experiments show that BC is able to increase iron uptake [35], making more iron available in the cells to react with H_2O_2. Moreover, BC and BCM seem to accumulate in the crude nuclei of NCI-H69 cells [36], being able to specifically react with DNA-associated transition metals to form $^{\cdot}OH$ in proximity to the DNA. The formation of $^{\cdot}OH$ in a reaction of H_2O_2 with Fe^{2+} was indeed increased by BC, BCM, and Vit E (Fig. 6, step 3), which might be attributed to the ability of BC, BCM, and Vit E to reduce iron from Fe^{3+} to Fe^{2+}, making it better available for electron transfer with H_2O_2 [37].

We observed an inverse correlation between MPO activity and the level of oxidized purines, suggesting that decreased MPO activity leads to increased H_2O_2 and thus to increased formation of oxidized purines. Because H_2O_2 plays a crucial role in the formation of neutrophil induced oxidized DNA, it would be interesting to measure the actual H_2O_2 concentration. Unfortunately, most described methods to measure H_2O_2 use iron or iron-containing enzymes like horseradish peroxidase, which are affected by BC (data not shown), making these methods unsuitable for the measurement of H_2O_2 in systems containing BC. For that reason, no H_2O_2 was assessed in the present study.

BC concentrations that were used in our study were high but physiologically achievable, because similar BC serum concentrations were accomplished after 3 years of daily BC intake, based on the ATBC and CARET studies. In the CARET study, the average serum Vit A level was 2.2 μM, both in the placebo and in the intervention group [38]. However, Vit A levels in serum are highly regulated. In contrast, Vit A levels in lung are less well regulated and do not correlate with serum Vit A levels [39], but they do correlate with the concentrations observed in bronchoalveolar lavages (BAL). Vit A levels in BAL were assessed in a study using five CARET participants (four intervention and one placebo) and twenty-one additional subjects participating in a study performed like the CARET, but with an intervention period of only 6 months. The additional intervention study indicated a slight increase in Vit A levels in BAL after 6 months of intervention, from 0.5 to 0.7 μM, whereas the BAL Vit A level in CARET participants after 5 years of intervention was 1.9 μM [39,40]. These data indicate that Vit A increases in lung cells upon supplementation, which is also supported by data in the mouse [41]. RA concentrations were not measured in the ATBC and CARET studies. The highest used concentration of RA may be physiologically high, because RA concentrations in plasma are in the nanomolar range [42] However, higher levels of RA are usually found in tissues by endogenous oxidation of Vit A [43], especially in the lung [44]. Overall, the BCM (10 μM) concentrations that we used in our in vitro experiments are relatively high but physiological relevant.

In addition to the BCM investigated in our study, other BC-derived products are known, which are generated by various mechanisms such as autoxidation and radical reactions [45]. Siems and colleagues demonstrated an important role for stimulated neutrophils in oxidative BC breakdown [46]. These BCM may have, similar to Vit A and RA, inhibitory effects on MPO activity. As a follow-up to our study, it would be of interest to further examine other BCM and possibly also Vit E metabolites, and interactions between different antioxidant vitamins and their breakdown products [47,48].

In conclusion, we demonstrate a role for BCM in neutrophil-induced genotoxicity. The BCM Vit A and RA were able to decrease MPO activity and enhance $^{\cdot}OH$ radical formation in the Fenton

reaction. This ultimately led to an increase in the level of oxidized purines in the DNA of lung epithelial cells when cocultured with freshly isolated activated neutrophils. Further research is needed to verify the relevance of these effects for neutrophil-induced genotoxicity in the lung in vivo.

Acknowledgments

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