BRIEF COMMUNICATION

No Influence of Beta Carotene on Oxidative DNA Damage in Male Smokers

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Several large-scale human intervention studies are currently evaluating whether beta-carotene supplements may protect against cancer (*1*). A plausible mechanism for beta carotene could be its ability to scavenge reactive oxygen species that cause oxidative DNA damage, a crucial event in carcinogenesis (*2*). The most abundant base alteration induced in DNA by reactive oxygen species is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) (*3*). In vivo this DNA base alteration is repaired by excision, and the resulting product, 8-oxodG, is excreted unchanged and independently of diet into the urine. The rate of excretion of 8-oxodG thus serves as a biomarker of the integrated rate of oxidative DNA damage in the whole body (*4*). The 8-oxodG measure has been used to demonstrate 50% higher rates of oxidative DNA damage in smokers, who have a known increased risk of cancer (*4*). Recently, beta carotene came into debate after a large trial in Finnish smokers did not report fewer, but rather reported even more, lung cancers following treatment with beta-carotene supplements (*5*).

In the present study, we have examined in a randomized intervention trial the hypothesis that supplementary beta carotene results in a reduction of oxidative DNA damage in male cigarette smokers.

The design of this trial was described previously (*6*). Briefly, volunteer smokers of more than 15 cigarettes per day were randomly assigned to receive either beta carotene (20 mg/day; Hoffmann-La Roche, Basel, Switzerland) or placebo treatment (i.e., lactose) for 14 weeks. The trial was approved by the external TNO Medical Ethical Committee, and each participant provided written informed consent. A total of 163 smokers (83 in the placebo group and 80 in the beta-carotene group) volunteered to participate. During the trial, 13 smokers terminated participation. Blood samples were collected from all participants before and after the treatment. During week 14, urine samples were collected for 3 consecutive days, excluding working hours (i.e., approximately 9 AM to 5 PM). Blood levels were measured before and after treatment for vitamin C, retinol, α-tocopherol, beta carotene, and cotinine (*6*). The concentration of 8-oxodG was measured in urine samples collected in week 14 by high-performance liquid chromatography with electrochemical detection as described previously (*4*). In 25 subjects, interfering chromatographic peaks that may have been related to recent intake of paracetamol (*4*) precluded analyses. In three subjects, information on creatinine analyses was missing, leaving 122 subjects (57 placebo treated and 65 beta carotene treated) for the analyses reported here.

The placebo and beta-carotene groups had comparable ages (means ± SD = 39.0 ± 10.0 years for the placebo group versus 39.3 ± 9.1 years for the beta-carotene group), body mass index (24.5 ± 2.7 kg/m^2 versus 24.6 ± 3.1 kg/m^2), and smoking habits (20.7 ± 5.8 cigarettes per day versus 21.4 ± 5.8 cigarettes per day; 20.7 ± 10.1 years of smoking versus 21.1 ± 9.3 years of smoking) and also had similar biochemical characteristics during the trial (Table 1). Plasma cotinine levels reflect stable smoking habits. The excretion of 8-oxodG (mean ± SD) after the 14 weeks was almost identical in the placebo and the beta-carotene groups, whether expressed as nanomoles per mole creatinine (2.83 ± 1.18 versus 2.98 ± 1.05, i.e., 5% higher in the beta-carotene group, 95% confidence interval, −9% to +19%) (Fig. 1) or as total 8-oxodG excreted during the three consecutive periods (67.66 ± 32.71 nmol versus 62.25 ± 28.54 nmol).

This trial in heavy smokers shows no effect of beta carotene on oxidative DNA damage, as assessed by 8-oxodG excretion. We did not measure 8-oxodG excretion at the beginning of the trial, but an initial difference seems improbable in our randomized design. The dose of beta carotene in this study is similar to the doses in the ongoing trials (*1,5*). A dose of 20 mg beta carotene per day is five to 10 times the normal intake, and plasma levels of beta carotene increased 14-fold (Table 1). Moreover, even after we excluded supplement-treated subjects with plasma beta-carotene values below the median (4.13 μmol/L), 8-oxodG excretion was still 8% higher (95% confidence interval, −12% to +23%). Our results indicate that there is only a 5% chance of a 7% or more reduction in 8-oxodG in the beta-carotene group.

Beta carotene has been shown to function as an antioxidant in many, but not all, in vitro systems (*7*). Our results suggest that beta carotene does not act as an in vivo antioxidant reducing oxidative DNA damage in humans. In smokers, beta carotene has been reported to diminish breath pentane as an index of lipid peroxidation (*8*), but not low-density lipoprotein oxidizability (*9,10*). Other antioxidants may explain the inverse associations between vegetables and cancer risk. We recently demonstrated a ±30% reduction in 8-oxodG excretion after nonsmoking volunteers had consumed 300 g of Brussels sprouts for 3 weeks (*11*).

Our study is in line with the lack of an effect of beta carotene on lung cancer reported in Finland (*5*) and on colorectal adenoma reported in the United States (*12*). It is tempting to speculate that our

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See "Notes" section following "References."

Table 1. Initial and final values (means ± SD) of blood parameters during a 14-week intervention trial in male smokers, assigned to treatment with either beta carotene or placebo

	Placebo group (n = 57)		Beta-carotene group (n = 65)	
	Initial values	Final values	Initial values	Final values
Blood vitamin C, μmol/L*	37.2 ± 19.5	38.5 ± 17.5	36.9 ± 17.4	34.8 ± 16.6
Plasma retinol, μmol/L	2.28 ± 0.42	2.23 ± 0.43	2.35 ± 0.49	2.40 ± 0.59
Plasma α-tocopherol, μmol/L	30.8 ± 6.9	32.3 ± 6.7	31.4 ± 6.4	31.8 ± 6.3
Plasma beta carotene, μmol/L	0.28 ± 0.18	0.26 ± 0.14	0.31 ± 0.15	4.25 ± 2.28†
Plasma cotinine, μg/L	314.2 ± 113.8	307.4 ± 129.1	336.9 ± 106.4	319.9 ± 102.1

*Six missing values.
†Beta-carotene group was significantly different from placebo group, $P<.0001$.

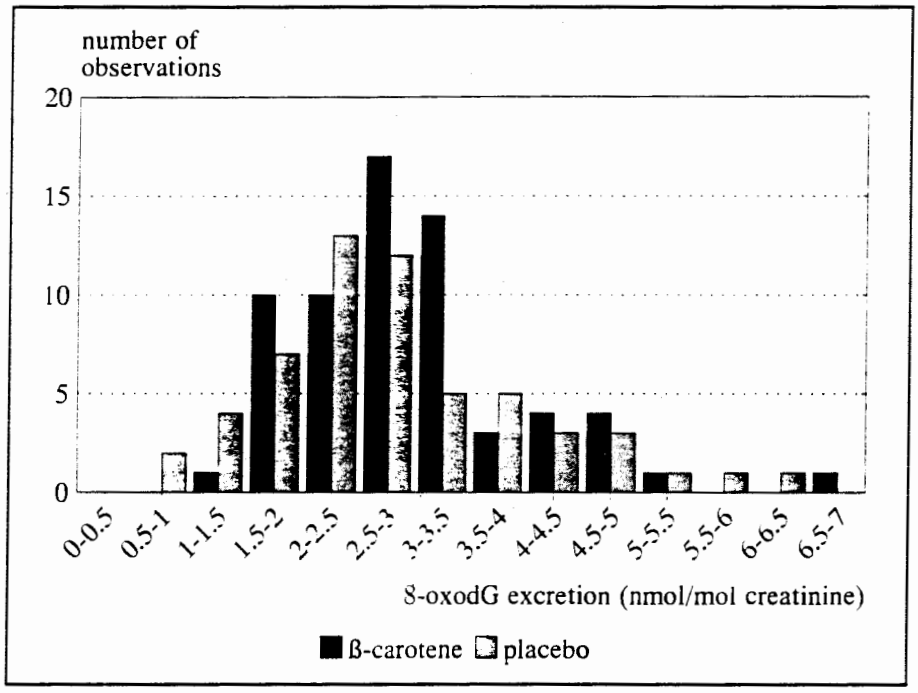

Fig. 1. Urinary 8-oxodG excretion in smokers after 14 weeks of beta-carotene treatment (20 mg/day; n = 65) or placebo treatment (n = 57).

study provides further evidence of the absence of a cancer preventive potential of beta carotene. However, the predictive value of the 8-oxodG measure for cancer development has not been established. Other studies (*1,6*) have shown beneficial effects of beta carotene on micronuclei in expectorated sputum cells and buccal mucosal cells and on oral leukoplakia. Also, a combination of beta carotene, vitamin E, and selenium reduced stomach cancer mortality in marginally nourished people in Linxian, China (*13*). Protective mechanisms for beta carotene that do not involve oxidative DNA damage may include in situ conversion to retinoids and effects on gap-junction communication and on metabolism of carcinogens, as well as immunomodulatory effects (*1*). Such a mechanism not involving oxidative DNA damage may explain the reduction of sputum micronuclei after beta-carotene supplementation in this same study population (*6*). We did not observe a correlation between 8-oxodG excretion and sputum micronuclei ($r = -.035$).

Our results using the 8-oxodG measure do not support the hypothesis that beta carotene affects cancer risk by preventing oxidative DNA damage in humans. It cannot be excluded, however, that beta carotene affects other forms of DNA damage or has other preventive mechanisms. Data from the ongoing large trials will eventually provide further information on the potential benefits of beta carotene.

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Notes

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