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Antimutagenic Activity of β-Carotene

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Data are presented on the ability of synthetic β-carotene to reduce the level of cyclophosphane-induced chromosome aberrations in murine bone marrow cells. In *Salmonella typhimurium* cells β-carotene exhibits antimutagenic activity only against an indirect mutagen (2-acetylaminofluorene).

Key Words: *β-carotene; antimutagenic activity; chromosome mutations; Ames test*

The efficacy of β-carotene (BC) as an oncoprophylactic agent has been proven in epidemiological trials which demonstrated a correlation between the plasma carotenoid level and cancer morbidity. There are some data on the preventive effect of BC added to food on the induction of tumors by chemical agents in rodents [4]. It is currently believed that the first stage of carcinogenesis (initiation) consists in the induction of mutations by various agents which results in the activation of cell protooncogenes and in the formation of a primary tumor cell. Hence, an analysis of the antimutagenic properties of BC is very important for understanding its antitumor effect. The aim of our study was thus to investigate the antimutagenic properties of BC using *in vivo* and *in vitro* models.

It should be noted that little is known about the methodology of studies of the antimutagenic effect of BC. The approach commonly used is one whereby: the antimutagenic effect of BC is studied in short-term mutagen tests with known inductors in optimal concentrations.

Experiments on mice showed that BC in large doses rapidly reduced the number of chromosome aberrations (CA) induced by direct mutagens [7,8]. BC lowered the frequency of micronucleation in murine bone marrow cells induced by mitomycin C and benzo(a)pyrene. The specificity of the effect of BC was found in cultured CHO cells: when added to the culture medium, it reliably lowered the level of CA induced by directly acting mutagens but was inactive against others [10]. Since little has been reported on the antimutagenic effect of BC, established [1,6,9] and not established [1,9,11] in the Ames test, it seemed important to study this effect using modeled gene mutations in microbial cells.

MATERIALS AND METHODS

For the *in vivo* model we used the cytogenetic test which counts cyclophosphane (CP)-induced CA in murine bone marrow cells. Synthetic BC (*Vitaminy* Research-and-Manufacturing Plant) was applied in a 30% paste also supplemented with antioxidant. The paste was dissolved in olive oil and given to 6-month-old CBA male mice weighing 22-25 g (10 mg/kg body weight BC daily during 10-12 days). A paste containing antioxidant and olive oil served as control. CA were induced by intraperitoneal injection of CP (30 and 60 mg/kg body weight). Metaphases were analyzed after 24 h, i.e., during the first wave of mitoses (at least 50 metaphase plates

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TABLE 1. Effect of BC on Induction of CA ($M \pm m$)

Experimental conditions	Total number of metaphase plates	Metaphase plates with CA, %
CP, 3 mg/kg		
Control	100	39±4.8
Supplements	150	20±3.3*
BC	100	11±3.1*
CP, 60 mg/kg		
Control	150	64±3.9
Supplements	100	31±4.6*
BC	200	21±2.9*
Without CP		
Control	157	3.2±1.36
BC	100	2±1.3***

Note. Here and in Table 2: *$p<0.01$, **$p<0.05$, ***$p<0.1$ in comparison with the respective control.

per mouse were counted). In the analysis of structural chromosome abnormalities we counted chromatid and isochromatid breaks and translocations; genes were counted separately and their number was not entered in the general table.

For the *in vitro* model the classical Ames test was used. Gene mutations were induced in *Salmonella typhimurium* TA 98 and TA 100 by chemicals of two types: directly acting agents (thiophosphamide, pyrene derivative, nitrulline) and agents requiring preactivation in the presence of a microsomal enzyme mixture consisting of mouse liver homogenate and cofactors. The inductors of activation phenobarbital and sovol were used for the indirect mutagens 2-acetylaminofluorene and benzo(a)pyrene. Synthetic crystalline BC was dissolved in acetone (100 μl/dish) and used in the maximal available concentration of 18 μg/dish (spectrophotometrically determined).

In the statistical processing of the results the reliability of differences between the mean values was determined using the Student t test.

RESULTS

The data presented in Table 1 show that BC in this medicinal form (oil paste of synthetic preparation) suppressed CP-induced CA. The medicinal base containing antioxidant supplements reduced the percentage of induced metaphase plates 2-fold. Addition of BC to food enhanced the protective effect, the percentage of induced CA decreasing 3-4-fold depending on the dose of CP. It is important to note that CP caused a relatively large number of multiple breaks and translocations. Against the background of treatment with BC these serious chromosome abnormalities were usually absent, while single-strand breaks prevailed among CA. When this study had been completed, a report appeared [5] that BC in doses of 2.7-27 mg/kg body weight added to food decreased the number of CP-induced CA 1.5-2-fold. This fully coincides with the parameters of our model and with the results.

TABLE 2. Effect of BC on Induction of Gene Mutations

Experimental conditions		Inhibition of His^+/revertants, %
2-acetylaminofluorene, μg/dish	microsome enzyme mixture, ml/dish	
8	0.6	24*
8	0.5	27***
8	0.3	34**
25	0.3	18***

In the Ames test BC exhibited marked antimutagenic activity only against the indirect mutagen 2-acetylaminofluorene. This activity was directly proportional to the dose of the mutagen and inversely proportional to the amount of microsomal enzyme mixture (Table 2). No such protective effect was observed in a preliminary experiment with benzo(a)pyrene (5 and 10 μg/dish), which is consistent with previous data [11]. The positive effect of BC in experiments with CP [1] (which requires an inductor of the microsomal enzyme system common to 2-acetylaminofluorene) implies its relationship with retinol [6] with respect to inhibitory effects against mutagens activated by certain forms of cytochrome P-450. Not one modification of the Ames test revealed an effect of BC on the mutagenicity of the three direct mutagens, which is in conformity with previous data [1,9]. The protective effect observed by us may have resulted from an extracellular suppression of the microsomal activation system, and this *in vitro* model evidently cannot be used for the study of an antioxidant protective effect observed *in vivo*.

For a better understanding of the mechanism of the *in vivo* antimutagenic effect we should note that BC has been reported to reduce the number of CA induced by direct mutagens in Chinese hamster bone marrow cells. The effect was observed only when the preparation was administered 2 hours prior to the inductor [8], but no protective effect was observed with CP [7]. As follows from the comparison of these data with our findings, BC evidently acts as a dismutagen (interceptor) of a directly acting genotoxic agent at the time of injection. For BC to manifest its full antimutagenic effect against indirect mutagens, on the other hand, a longer period of its utilization (or, possibly, metabolism) is required. This is presumably accomplished through inhibition of the induced

form of cytochrome P-450 which is responsible for transforming the mutagen into active forms.

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