EFFECT OF β-CAROTENE ON TOXIC GENETIC INJURIES IN THE LIVER DURING FORMATION AND ACTION OF THE CARCINOGEN N-NITROSODIMETHYLAMINE

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There is much evidence in the literature that retinoids can inhibit the development of experimental tumors in animals. β-Carotene can inhibit tumor development induced by dimethylbenz(a) anthracene (DNBA) [13] and also the development of skin tumors induced by ultraviolet rays [10].

Epidemiologic studies have demonstrated negative correlation between the β-carotene content in the human diet and the incidence of cancer [11]. It has been shown that β-carotene acts both on the initiation phase and on the promotion phase in carcinogenesis induced by DNBA [13]. We could find no data in the literature on the effect of β-carotene on carcinogenesis induced by N-nitrosodimethylamine (NDMA).

The aim of this investigation was to study the effect of β-carotene on toxic genetic injuries in the liver arising during both endogenous synthesis and exogenous intake of NDMA. Changes in enzyme activity due to the toxic action of the carcinogen on the liver cells also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-200 g.

To study alkylation of DNA (experiments of series I) we used ^{14}C-NDMA with specific activity of 344 MBq/mmole and ^{14}C-dimethylamine (^{14}C-DMA) with specific activity of 2.15 GBq/mmole (from "Amersham International," Great Britain). To obtain the necessary dose of ^{14}C-DMA, unlabeled DMA was added, so that the final specific activity of the solution was 60 MBq/mmole. In the experiments of series II unlabeled NDMA and precursors for its synthesis — amidopyrine (AP) and sodium nitrite, — and also β-carotene, dissolved and stabilized in refined sunflower oil (3 mg/kg) were used.

Animals in the experiments of series I were divided into four groups, with 4-7 animals in each group. Animals of group 1 were given ^{14}C-NDMA in a dose of 8 mg/kg, animals of group 2 received ^{14}C-DMA (500 mg/kg) together with sodium nitrite ($NaNo_2$; 125 mg/kg); animals of groups 3 and 4 received β-carotene in a dose of 3 mg/kg daily for 5 days, and 2 h after the last dose they were given ^{14}C-NDMA or ^{14}C-DMA + $NaNO_2$ respectively.

In the experiments of series II the animals were divided into five groups (10-12 in each group). The rats of group 1 received NDMA (8 mg/kg) and those of group 2 received AP (250 mg/kg) together with $NaNO_2$ (125 mg/kg). Animals of groups 3 and 4 received β-carotene in the same way as in the experiments of series I, after which they were given NDMA or AP + $NaNO_2$ respectively. Animals of group 5 were the intact control. All solutions were administered by tube.

The animals were killed under ether anesthesia 24 h after administration of the carcinogen or precursors. The level of alkylation of purine bases of DNA in the liver [9] and the formation of single-stranded DNA breaks [1] were determined in the liver and activity of γ-glutamyl-

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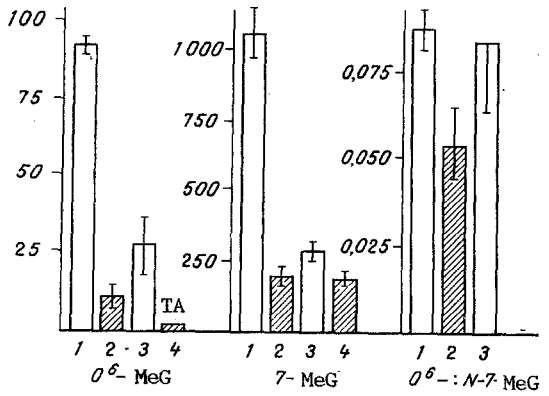

Fig. 1. Effect of β-carotene on methylation of DNA during formation and action of NDMA. Abscissa: 1) ^{14}C-NDMA, 2) β-carotene + ^{14}C-NDMA, 3) ^{14}C-DMA + $NaNo_2$, 4) β-carotene + ^{14}C-DMA + $NaNO_2$; ordinate, quantity of alkylated base of DNA, μmoles/mole guanine. TA) Trace amounts.

TABLE 1. Effect of β-Carotene on Formation of Single-Stranded Breaks in DNA and on Enzyme Activity during Formation and Action of NDMA (M±m)

Treatment	Quantity of single-stranded DNA as a ratio of total DNA, %	Enzyme activity		
		AlT	SDH	γ-Glutamyl-transpeptidase
Control	6,64±0,31	1,04±0,08	0,46±0,05	0,87±0,03
NDMA	25,65±0,23	1,53±0,07	3,17±0,43	1,50±0,01
β-Carotene + NDMA	15,10±0,94	1,29±0,06	1,53±0,17	0,90±0,02
AP + $NaNO_2$	21,68±2,30	1,73±0,07	1,58±0,07	1,32±0,05
β-Carotene + AP + $NaNO_2$	12,13±1,16	1,05±0,04	1,07±0,13	0,90±0,02

Legend. AlT activity expressed in mmoles pyruvic acid/h/liter; SDH activity in relative units ($51.6 \cdot \Delta A_{340}$, where A_{340} denotes optical density at 340 nm); γ-glutamyl-transpeptidase activity in pmoles 4-nitroaniline/min/mg protein.

transpeptidase was determined in the post-mitochondrial fraction [4]. Serum levels of alanine-aminotransferase (AlT) and sorbitol-dehydrogenase (SDH) activity were determined [4]. The results were subjected to statistical analysis with calculation of the arithmetic mean and its standard error by Student's test for small samples [7]. To study the level of DNA alkylation, the significance of the measurements was increased both by reducing the background value and by increasing the counting time, as determined with the aid of Bell's table [2].

EXPERIMENTAL RESULTS

Administration of both ^{14}C-NDMA and its precursors (^{14}C-DMA + $NaNO_2$) to the rats led (Fig. 1) to methylation of the liver DNA with the formation of 7-methylguanine (7-MeG) and of O^6-methylguanine (O^6-MeG). The mean values of radioactivity corresponding to the chromatographic peak of 7-MeG varied in the different groups from 2000 to 34,000 cpm, and the values for O^6-MeG were: 2940 cpm in group 1, 170-200 cpm in group 2, 260 cpm in group 3, and 85 cpm in group 4.

Preliminary treatment with β-carotene before the action of ^{14}C-NDMA reduced 7-MeG formation by 82% and O^6-MeG formation by 92%. In the case of endogenous NDMA synthesis from its precursors, β-carotene inhibited 7-MeG formation, but O^6-MeG was found in trace amounts. Lowering of the O^6-MeG: N-7-MeG ratio by 2.3 times may be evidence that the velocity of enzymic removal of O^6-MeG from the structure of DNA was higher in animals treated with β-carotene before the carcinogen.

The study of formation of single-stranded DNA breaks under the influence of exogenous and endogenous NDMA showed that their number was reduced by β-carotene by 55-63% (Table 1).

The toxic action of NDMA on liver cells was studied by determining changes in AlT and SDH activity (Table 1). Both exogenous NDMA and the sum total of the precursors increased activity of the above-mentioned enzymes in the animals' blood serum. Pretreatment of the rats with β-carotene significantly reduced the toxic action of the carcinogen, as shown by reduction of AlT and SDH activity. The action of NDMA caused a change in activity of γ-glutamyl-transpeptidase, which is known to be a marker of preneoplastic changes [8]. Since the short-term action of the carcinogen was investigated in the experiments described above, activity of this enzyme acted as a biochemical indicator of its toxic effect, which was completely abolished by β-carotene.

During the action of exogenous NDMA and during its synthesis from precursors, alkylation of DNA and the formation of single-stranded breaks thus take place [6]. The possibility cannot be ruled out that the high reactivity of β-carotene during interaction with different types of free radicals [3, 5] may be responsible for its protective effect against injury by NDMA. The latter was manifested as a decrease in enzyme activity, enhanced as a result of its toxic action.

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