Acta Biologica Hungarica 54 (1), pp. 89–93 (2003)

INVESTIGATION OF ETHYL ALCOHOL AND β-CAROTENE EFFECT ON TWO MODELS OF CARCINOGENESIS

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(Received: May 3, 2002; accepted: July 1, 2002)

The effects of ethyl alcohol and synthetic β-carotene have been studied on two models of carcinogenesis in mice BALB/c. Lung tumours were induced with organotropically acting urethane (given by i.p. injections, total dose – 100 mg/mouse), subcutaneous tumours were induced with locally acting benzo(a)pyrene (single injection, 2 mg/mouse). β-Carotene was given 3 times per week 0.4 mg/mouse by gastric intubations and 10% ethanol was given instead of drinking water until the end of experiments (4–6 months). Results showed that β-carotene did not significantly inhibit lung adenomogenesis and may moderately delay subcutaneous tumours occurrence. In our studies chronic ethanol intake did not show significant influence on this delay.

Keywords: Ethyl alcohol – β-carotene – chemical carcinogenesis – mice

INTRODUCTION

Epidemiological studies have suggested a role of dietary β-carotene in the risk-reduction of cancer [5, 7]. However, four major intervention studies did not provide evidence for a significant protective effect of β-carotene on risk of cancer [1]. On the other hand, CARET study showed an increased risk of lung cancer in heavy smokers and in those with higher alcohol intake. Dose, time of exposure, smoking status, imbalance of antioxidant defence may be factors which influence cancer risk. At present, however, there is no evident mechanism to explain how the interaction of β-carotene and alcohol might affect lung cancer, and this finding requires examination in various studies [1]. Recently, *in vitro* and *in vivo* studies showed that β-carotene itself may act as an anticarcinogen, but its oxidized products may facilitate carcinogenesis [7]. In studies in animal models of carcinogenesis β-carotene (given simultaneously or sequentially with various carcinogens by different doses, routes to mice, rats, hamsters) appeared to be protective (on skin, colon, pancreas, forestomach and other) or to be ineffective in preventing cancer (intestine, salivary gland, respiratory tract) [8].

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The aim of this study was to investigate possible influence of ethyl alcohol on anticarcinogenic properties of β-carotene in experiments on laboratory animals.

MATERIALS AND METHODS

Agents

β-Carotene, synthetic (Sigma), benzo(a)pyrene (BP) (Fluka), commercial urethane ("pure", Kiev, Ukraine) were used. Ethyl alcohol (EtA) was used in 10% solution.

Animals

BALB/c mice (8 weeks old, both sexes) were obtained from Institute of Immunology, Vilnius. The animals were cared for in accordance with European Convention, the Guide for care and use of laboratory animals and Lithuanian laws.

Experimental design

Exp. 1: 193 mice were divided in 6 groups: I gr. urethane (U); II gr. β-carotene + U; III gr. 10% EtA + U; IV gr. 10% EtA + β-carotene + U; V gr. β-carotene; VI gr. 10% EtA. U was given 10 mg/mouse twice a week by intraperitoneal injections (total dose – 100 mg/mouse). β-Carotene was given 0.4 mg/mouse in 0.1 ml olive oil 3 times a week intragastrically by gavage (starting 1 week prior to carcinogen administration and until the end of experiment). 10% EtA was given *per os ad libitum* (instead of drinking water). The body weight was recorded weekly. After 4 months all surviving mice were killed by cervical dislocation. Lungs were examined macroscopically and microscopically.

Exp. 2: 111 mice were divided in 4 groups: I gr. BP; II gr. β-carotene + BP; III gr. 10% EtA + BP; IV gr. 10% EtA + β-carotene + BP. BP was given 2 mg/mouse in 0.2 ml olive oil by single subcutaneous injection, β-carotene and EtA were given as described above in exp.1. After 58 weeks from the beginning of the experiment all mice still alive were killed and sacrificed. Tumours were removed, weighed and examined microscopically.

Histological examination

The tissues (lung, subcutaneous tumours, liver) were fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eozin. Mean number of tumours per tumour-bearing mouse, percentage of animals with tumours, time taken to develop tumours, histological type of tumours were considered.

Statistical analysis

Data were analysed statistically by Student's *t*-test.

RESULTS AND DISCUSSION

Table 1 shows the results on compounds currently being tested in the mouse lung adenoma assay.

U induced the tumours in every mouse given carcinogen. Pathological examination showed histological benign adenoma in lungs. β-Carotene used in low dose did not show inhibitory effect on lung tumours development: it was not significant change in tumour size, incidence or multiplicity. The lowest tumours number per mouse was in IV gr., but results were not statistically significant in comparison with I–III groups ($P > 0.05$).

According to the literature, β-carotene inhibited upper respiratory tumorigenesis in hamsters receiving diethylnitrosamine (DEN) followed by cigarette smoke exposure [3]. However, in several studies β-carotene did not show cancer-protective effects, for instance, it was not effective in lung carcinogenesis, induced with 4-nitro-

Table 1
Effect of ethyl alcohol and β-carotene on lung adenomogenesis induced with urethane in mice BALB/c

Groups No.	Treatment		Number of animals		Mice with tumours (%)	Lung adenomas/ mouse ±SD
			initial	effective		
1.	Urethane	%	20	17	17(100)	9.4±3.8
		&	20	20	20(100)	10.4±2.6
		Total	40	37	37(100)	9.9±3.2
2.	β-carotene +	%	20	19	19(100)	8.3±2.3
	Urethane	&	16	16	16(100)	10.1±2.4
		Total	36	35	35(100)	9.3±2.5
3.	10% ethyl alcohol +	%	20	20	20(100)	8.0±2.4
	Urethane	&	20	19	19(100)	8.4±2.6
		Total	40	39	39(100)	8.1±2.5
4.	10% ethyl alcohol +	%	20	20	20(100)	7.7±2.5
	β-carotene +	&	20	20	20(100)	6.9±2.8
	Urethane	Total	40	40	40(100)	7.3±2.7
5.	β-carotene	%	10	10	1(10)	0.1±0.3
		&	10	10	2(20)	0.3±0.7
		Total	20	20	3(15)	0.2±0.5
6.	10% ethyl alcohol	%	6	6	0(0)	0
		&	11	11	1(9)	0.1±0.3
		Total	17	17	1(6)	0.1±0.2

quinoline 1-oxide or BP in mice [4, 9]. In multiorgan carcinogenesis model β-carotene (similar dose as used in our experiment) reduced number of liver tumours, but had not inhibitory effect on lung tumours development, induced with DEN and N-methyl-N-nitrosourea in mice [6]. On the other hand, newly-developed form of β-carotene – liposomal β-carotene inhibited lung tumours in mice treated with U [2].

According to the literature, in almost all studies on skin carcinogenesis in mice, hamsters, β-carotene appeared to be cancer-preventive [8]. The results of our experiment showed that at 10 week after the beginning of treatment there were 20% of animals with tumour in group given only BP subcutaneously. At the same time there were no tumours in group β-carotene + BP. Till 3.8% animals with tumour were in groups 10% EtA + BP and 10% EtA + β-carotene + BP. At 18 week after the beginning of treatment the percent of animals with tumour was 60% (I gr.), 46.2% (II gr.), 46.1% (III gr.), 42.8% (IV gr.), respectively.

At the end of experiment the percent of animals with tumour was similar in all groups (accordingly 84.0%, 82.1%, 65.4%, 84.0%). Small differences observed were not statistically significant. The tumours were diagnosed histopathologically as subcutaneous fibrosarcomas. The weight of tumours was similar in the groups (13.8 g–14.8 g).

At autopsy no gross lesions related to the treatment were observed in liver and no remarkable changes were found under microscope.

In conclusion, synthetic β-carotene did not significantly inhibit urethane-induced lung adenomogenesis and may moderately delay BP-induced the occurrence of subcutaneous fibrosarcomas. Chronic 10% ethanol intake did not show significant influence on it under the present experimental conditions. The studies now continue to investigate the possible effect of ethyl alcohol on antigenotoxic properties of β-carotene *in vivo*. Preliminary results showed that β-carotene reduced the frequency of bone marrow micronuclei formation induced by BP in mice.

REFERENCES

1. Albanes, D., Heinonen, O. P., Taylor, P. R. (1996) α-Tocopherol and β-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of baseline characteristics and study compliance. *J. Natl. Cancer Inst. 88,* 560–570.
2. Aleksandrov, V. A., Bespalov, V. G., Petrov, A. S., Avtushenko, S. S. (1998) The inhibitory effect of water-soluble and liposomal beta-carotene on various models of carcinogenesis. *Vopr. Onkol. 44,* 79–85.
3. Furokawa, F., Nishikawa, A., Kasahara, K. et al. (1999) Inhibition by beta-carotene of upper respiratory tumorigenesis in hamsters receiving diethylnitrosamine followed by cigarette smoke exposure. *Japan J. Cancer Res. 90,* 154–161.
4. Murakoshi, M., Nishino, H., Satomi, Y. et al. (1992) Potent preventive action of alpha-carotene against carcinogenesis: spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice are suppressed more effectively by alpha-carotene than by beta-carotene. *Cancer Res. 52,* 6583–6587.
5. Pryor, W. A., Stahl, W., Rock, Ch. L. (2000) Beta carotene: from biochemistry to clinical trials. *Nutr. Rev. 58,* 39–47.

6. Tsuda, H., Iwahori, Y., Asamoto, M. et al. (1996) Demonstration of organotropic effects of chemopreventive agents in multiorgan carcinogenesis models. *IARC Sci. Publ. 139,* 143–150.
7. Wang, X. D., Russel, R. M. (1999) Procarcinogenic and anticarcinogenic effects of β-carotene. *Nutr. Rev. 57,* 263–272.
8. Woutersen, R. A., Wolterbeek, A. P. M., Appel, M. J. et al. (1999) Safety evaluation of synthetic β-carotene. *Critical Reviews in Toxicology 29,* 515–542.
9. Yun, T. K., Kim, S. H., Lee, Y. S. (1995) Trial of a new medium-term model using benzo(a)pyrene induced lung tumor in newborn mice. *Anticancer Res. 15,* 839–845.