

Initiation and post-initiation chemopreventive effects of β -carotene in toad liver carcinogenesis

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Summary. Hepatocellular carcinoma were recognized in the toad, *Bufo viridis*, in 14 cases out of 50 by injection of 1 mg 7,12 dimethylbenz(a)anthracene (DMBA)/toad twice/week for 12 weeks. In contrast, toads treated with DMB at the same dose level and β -carotene (β C), 0.05 mg (3 hr prior to the carcinogen)/toad, twice/week for 12 weeks showed no tumor incidence. However, β C at the same dose level was less effective when administered 3 hr. after the carcinogen (DMBA). In 8 cases out of 50 cases neither tumor growth nor neoplastic changes were observed in toads treated with β C alone or olive oil. It is concluded that β C completely blocked hepatocarcinogenesis in toads when given 3 hr. before initiation.

Key words: 7,12-Dimethylbenz(a)anthracene (DMBA), β -carotene (β C), Toad (*Bufo viridis*)

Introduction

It has recently become apparent that environmental factors play an important role in lowering the incidence of several types of human cancer (Weisburger, 1991). Retinoids have been shown to have anticarcinogenic activity in animal models (Hill and Grubbs, 1992; Sporn and Newton, 1979). Epidemiological investigations have shown that cancer risk is inversely related to the consumption of green and yellow vegetables (Peto et al., 1981; Hennekens et al., 1982). Since β -carotene (β C) is present in abundance in green and yellow vegetables and has the highest provitamin A activity, β C is proposed as a key cancer preventive agents. Besides serving as the major dietary source of retinol for humans, β C and other carotenoids have been found to possess common biological functions (photoprotection, antioxidant properties including singlet oxygen quenching, immunomodulation and anticancer activity) in both humans and rodents (Olson, 1989; Lim et al., 1992). It has been shown that β C prevented or delayed carcinogenesis

induced by chemicals (Santemaria et al., 1983; Alam and Alam, 1987).

Toads have been used as models to study the development of tumors in relation to carcinogen (El-Mofty et al., 1993), a co-carcinogen (Sadek and Abdul-Salam, 1994) and vitamins (Sadek, 1984). It is worth mentioning that similarities in cytological characteristics between tumors in frogs and human have been reported (Deyree et al., 1960).

Because the influence of retinoids on experimental hepatocarcinogenesis has received relatively little study, it is therefore of interest to examine the effect of β C on the incidence of liver tumor in toads, particularly when administered before initiation and post initiation of 7,12-dimethylbenz-(a)anthracene (DMBA) carcinogenesis.

Materials and methods

Sexually mature male and female toads, *Bufo viridis*, were used. The average weight per experimental animals was 30 g. The experimental animals were collected by a regular supplier from El-Wafra district, Kuwait. The toads were maintained in plastic tanks at a temperature of 20-22 °C and fed equal meal of earth worms, once per week. The experimental animals were divided into 5 groups (50 toads/group) and treated as follows:

1. The first group (group A), 23 male and 27 female, was injected with DMBA into the dorsal lymph sacs (Sigma Chemical Company, St. Louis, MO, USA) at a dose of 1 mg/toad, twice/week for 12 weeks.
2. Toads of group B (25 male and 25 female) were given the same dose level of DMBA and injected with 0.05 mg β C, 3 hr prior to the carcinogen, twice/week for 12 weeks.
3. Animals of group C (26 male and 24 female) were given in the same dose level of DMBA and β C, 3hr after the carcinogen twice/week for the same period.
4. Group D (22 male and 28 female) was injected with β C alone at 0.05 mg/toad, twice/week for 12 weeks.
5. Toads of group E, 24 male and 26 female, were given 0.05 ml of olive oil and used as control.

At the end of 12 weeks, all animals were killed and all organs including the liver were carefully examined

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Table 1. Effect of β -carotene on toad liver tumor induced by DMBA.

GROUP	TREATMENT	DOSE	TOTAL NUMBER OF TOADS		NUMBER OF TOADS BEARING LIVER TUMOR		TOTAL NUMBER OF TOADS BEARING LIVER TUMOR	PERCENTAGE (%)
A	DMBA	1 mg	23 (3)	27 (3)	6	8	14	32
B	DMBA+ β C 3 hr before	1 mg+0.05 mg	25 (1)	25 (1)	0	0	0**	0
C	DMBA+ β C 3 hr after	1 mg+0.05 mg	26 (2)	24 (2)	4	4	8*	17
D	β C	0.05 mg	22	28 (1)	0	0	0	0
E	Olive oil	0.05 mg	24	26	0	0	0	0

(): number of dead toads. *: significant $p < 0.056$, as compared with DMBA group alone; **: highly significant $p < 0.0001$, as compared with DMBA group alone.

macroscopically. Tumors appeared in the liver of some animals. These tumors were greyish-white in colour. For the histological evaluation the liver tissue was fixed in Bouin and embedded in paraffin. The sections were stained with hematoxylin and eosin.

Statistical analysis for χ^2 test was done according to Steel and Torrie (1960) to determine the level of significant differences between tumor incidence of β C on tumor in toads treated with DMBA alone when compared with toads treated with DMBA and β C.

Results

Liver tumor was recognized in toads which had received 1 mg of DMBA/toad, twice/week for 12 weeks. This resulted in a tumor incidence of 32 percent (Table 1). Liver tumor incidences were diagnosed as hepatocellular carcinoma (Fig. 1a,b). The tumor cells showed all criteria of malignancy such as pleomorphic changes, hyperchromatinism and loss of polarity.

In the experimental toads treated with DMBA at the

same dose level and 0.05 mg of β C 3 hr prior to the carcinogen, twice/week for 12 weeks the carcinogenic effect of DMBA was completely blocked ($p < 0.0001$).

Toads treated with DMBA at the same dose level and 0.05 mg of β C 3 hr after the carcinogen, twice/week for 12 weeks (16 percent) showed a lower incidence of liver tumors which was nearly significant ($p < 0.056$).

Neither tumor growth nor neoplastic changes were detected after 12 weeks in the liver of toads which were given 0.05 mg of β C/toad, twice/week. Also, no tumor incidences were detected in toads which were treated with 0.05 ml of olive oil and used as control.

Discussion

β -carotene, a common constituent of carrots and green vegetables, has been proved to be a powerful antioxidant «provitamin A». It has been shown to have profound protective actions against carcinomas in skin, colon, buccal pouch epithelia, mammary gland, salivary gland and liver in experimental animals (Mathews-Roth, 1982; Schwartz and Shklar, 1988; Morene et al., 1991). A few studies have been conducted to determine the chemopreventive efficacy of carotenoids on liver carcinogenesis. Carrot feeding resulted in a significant delay in hepatoma formation in rats treated with diethylnitrosourea (DEN) (Rieder et al., 1983). The preneoplastic lesions induced by DEN in resistant

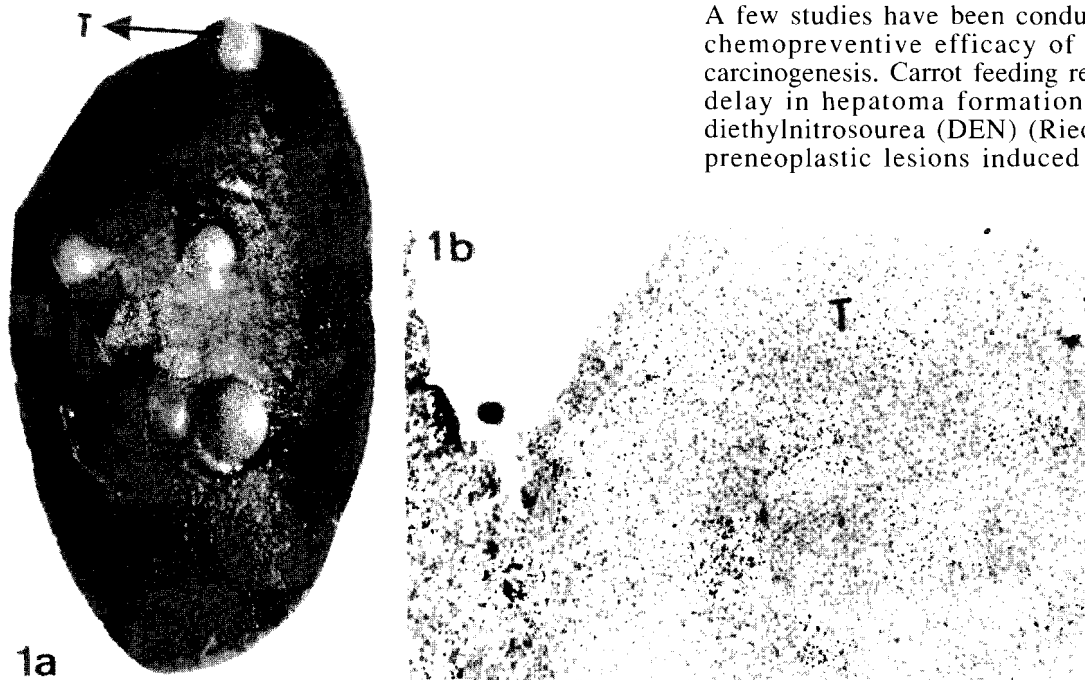


Fig. 1. a. Liver of toad treated with 7,12-dimethylbenz(a)-anthracene (DMBA) with tumor (T). b. Portion of a section of liver of a toad treated with DMBA. Note tumor (T) which was diagnosed as hepatocellular carcinoma. x 400

hepatocyte models in rat have been found to be decreased to a statistically significant level by β C (Morene et al., 1991).

On the other hand, Moon (1989) reported no effect of β C on N-butyl-N(4-hydroxybutyl) nitrosamine (OH-BBN)-induced bladder carcinogenesis. Similarly, no discernible effects on the growth-rate of Morris transplantable hepatoma could be observed following dietary administration of β C for 6 weeks (Blakely et al., 1988).

The results of the present investigation suggest that β C inhibited completely toad liver tumor when administered before initiation of DMBA carcinogenesis, and that it was less effective when administered after the carcinogen.

Although retinoids and carotenoids can inhibit both initiation and promotion of carcinogenesis (Moon and Mehta, 1986; Suda et al., 1986), it has been noted that they seem to block the development of neoplastic events primarily at the promotional phase (Krinsky, 1989). Furthermore, the inhibition of transformation of whole mammary glands from BALB/c female mice was maximum in glands treated concurrently in vitro with β C and DMBA, suggesting, therefore, that the preventive action of β C was highly potent during the initiation stage of the transformation process (Som et al., 1984).

The mechanisms by which β C inhibits liver tumor in initiation or post initiation are unknown. There is increasing interest in the role of antioxidant vitamins like ascorbic acid, α -tocopherol, retinol and β C in neutralizing free radicals and overtly aggressive oxygen species (Burton and Ingold, 1984). Free radicals and non-radical oxidizing species are regularly produced in animals treated with carcinogens (Sato, 1990) and also in human tissues (Ames, 1987) which are capable of damaging DNA, proteins and lipids. Furthermore, β C is among the most efficient substance known for quenching the excitation energy of single oxygen and also for trapping certain organic free radicals. It has a direct inhibitory effect on liver microsomal enzymes (Basu et al., 1987), thus offering another mechanism of its anti-cancer nature.

It is concluded that the time of dosing with β C is often important. To be most effective in preventing toad liver tumor, β C should be applied shortly before initiation. Much additional study is required to determine the relationship between β C and immune system, but clearly amphibians have a well developed immune system that consists of major cellular and humoral components (Cooper, 1976). Frogs possess cytotoxic cells which appear functionally similar to mammalian natural killer cells (Ghoneum et al., 1990). How these cells may prevent the development of neoplasm must still be subjected to experimentation.

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