# β-Carotene-Containing Preparation Carinat Inhibits Lipid Peroxidation and Development of Renal Tumors in Rats Treated with Chemical Carcinogen

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The effects of pretreatment with β-carotene-containing preparation carinat on the development of renal tumors in rats receiving single intravenous injection of chemical carcinogen 3-(1-α-L-arabinopyranosyl)-1-methyl-1-nitrosourea were studied. Fourteen months after carcinogen administration, the degree of lipid oxidation in rat kidneys 2.5-fold surpassed that in animals receiving carinat in a dose producing *in vivo* antioxidant effect. Carinat decreased the total number of induced tumors and the incidence of mesenchymal renal tumors and suppressed the development of multiple tumors. The accumulation of lipoperoxides in the kidneys during carcinogenesis is associated with activation of free radical processes and carcinogen-induced inhibition of lipoperoxide enzymatic degradation and probably promotes renal malignancies due to co-carcinogenic action of these compounds. The data suggest that carinat-induced suppression of tumor development attests to antioxidant effects of β-carotene.

**Key Words:** *antioxidants; lipoperoxides; β-carotene; chemical carcinogenesis*

The growth of malignant tumors is accompanied by marked changes in lipid metabolism due to intense lipid transport into the growing neoplasm [1,12]. Chemical carcinogens impair the regulation of free radical processes not only in the tumor, but also in normal tissues [2,3]. It was shown that malignant cells are resistant to free radical oxidation of endogenous unsaturated lipids [4,6,10]. However, the rate of free radical lipid oxidation and the concentration of lipoperoxides increase in various tissues of animals with transplanted or carcinogen-induced tumors, especially during rapid tumor growth [2,3,6]. It can be suggested that accumulation of toxic lipoperoxides in tumor tissues contributes to cachexia accompanying malignant growth [3,6]. In addition, lipoperoxides produce co-carcinogenic effects, *e.g.*, potentiate the action of true carcinogens [14]. Probably, antioxidants can decrease the rate of tumor growth and prolong the lifespan. Studies of various antioxidants, in particular of β-carotene, produced encouraging results [9,15]. At the same time, high doses of β-carotene stimulate malignant growth probably due to the concentration-dependent inversion of its antioxidant effects *in vivo* related to the formation of free radical intermediates of provitamin A during oxidation of the isoprenoid chain [5]. Here we studied the effects of β-carotene-containing preparation carinat on the development of tumors caused by single injection of chemical carcinogen 3-(1-α-L-arabinopyranosyl)-1-methyl-1-nitrosourea (AMNU).

## MATERIALS AND METHODS

Experiments were performed on female outbred albino rats weighing 155±5 g and fed standard diet. Groups 1 ($n$=20) and 2 ($n$=25) rats received single intravenous injection of 250 mg/kg AMNU (N. N. Blokhin Rus-

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sian Oncology Research Center) [8]. Groups 2 and 3 intact rats ($n$=20) daily received (through a gastric tube) 0.5 ml water suspension of β-carotene-containing nutriceutic carinat (Inat-Farma) in a dose equivalent to 0.4 mg/kg β-carotene over 1 month before and 14 month after AMNU injection [7]. The dose of carinat was monthly corrected for body weight gain. Group 1 rats received (through a gastric tube) 0.5 ml distilled water. Fourteen months later, survivors were euthanized, dissected, and examined macroscopically. Tumor samples were fixed in neutral formalin for histological assay. Tissue samples were embedded in paraffin, stained with hematoxylin and eosin, and examined under a microscope. Since AMNU causes primarily kidney tumors [8], renal tissue without visible signs of carcinogenesis was taken for biochemical studies. In case of extensive malignant lesions of the kidney, the samples were taken from another kidney without tumor infiltration. The kidneys (0.1 g wet tissue/ml) were homogenized in cold 0.154 M NaCl using an Ultra-Turrax SDT-1810 homogenizer (Tekmar). Lipids were extracted with chloroform-methanol mixture (2:1, v/v) by the method of Folch. Chloroform was evaporated in vacuum and then in an argon flow to a constant weight. The content of lipids was measured gravimetrically. Lipids were dissolved in methanol, and the content of lipoperoxides was estimated by oxidation of $Fe^{2+}$ to $Fe^{3+}$. The concentration of $Fe^{3+}$ before and after reduction of organic hydroperoxides with triphenylphosphine was measured by the reaction with xylenol orange at 560 nm on a Hitachi 557 spectrophotometer [13]. The results were analyzed by $\chi^2$ test for small samples (Yates correction) [8].

## RESULTS

AMNU having toxic properties caused death of 1 rat in group 1 and 2 rats in group 2 within 2-3 days postinjection. Examination revealed degenerative changes in parenchymal organs (numerous point hemorrhages). The rate of tumor growth did not differ between rats of groups 1 and 2 over 14 months of observations. In rats of groups 1 and 2 died before euthanasia, the first tumor node was found 238 and 245 days postinjection, respectively. The mean latencies of tumor development in these animals were 264±20 and 273±20 days, respectively. The incidence of tumor development in group 2 rats was 1.4 times lower than in group 1 rats. At the same time, the number of mesenchymal renal tumors in group 1 rats 1.5-fold surpassed that in group 2 (Table 1). The number of animals with multiple tumors (mesenchymal renal tumors and sarcomas of the large and small intestines and subcutaneous fat) significantly differed between groups 1 and 2. Multiple tumors were not found in group 2 rats, while 30% animals in group 1 had not only renal tumors, but also 1-2 tumors of other histogenesis and localization (Table 1). Hence, carinat considerably inhibits chemical carcinogenesis induced by AMNU. The content of lipid hydroperoxides in the kidneys of group 1 rats 2.5-fold surpassed that in group 2 animals (Table 1). It should be emphasized that the concentration of lipid hydroperoxides in the kidneys of group 2 rats was comparable with that in group 3 animals (Table 1). A sharp increase in the content of lipoperoxides caused by AMNU can be explained by the fact that this compound (similarly to other N-nitroso-N-methylurea derivatives) inhibits glutathione reductase during early carcinogenesis, thus blocking enzymatic degradation of lipoperoxides [1] and intensifying free radical reactions. β-Carotene was administered in doses stimulating the antioxidant system in rat tissues (as demonstrated in our previous experiments with carinat and β-carotene) [5,7]. Hence, our experiments showed that carinat prevents free radical lipid oxidation in rat kidneys induced by carcinogens and, therefore, attenuates their carcinogenic effects in target organs. Since lipoperoxides are co-carcinogens [14], their accumulation in the kidneys predisposed to AMNU-induced malignancy enhances the action of this carcinogen. Antioxidants should suppress the development of AMNU-induced tumors by inhibiting lipid peroxidation in the kidneys. In conclusion, pretreatment with β-carotene (carinat) in a dose producing *in vivo* antioxidant effect

**TABLE 1.** Effects of Carinat Treatment for 14 Months on Tumor Growth and Lipid Peroxidation in the Kidneys of Rats Receiving Single Intravenous Injection of AMNU

| Parameter | AMNU | AMNU+carinat | Carinat |
|---|---|---|---|
| Total number of rats with tumors, %* | 62.5 | 44.4 | 0 |
| Number of rats with mesenchymal renal tumors, %* | 56.2 | 38.8 | 0 |
| Number of rats with multiple tumors, %+ | 30.0 | 0$^{x}$ | 0 |
| Content of lipid hydroperoxides, nmol/mg lipids | 11.7±0.9 | 4.5±0.6$^{x}$ | 4.8±0.6 |

**Note.** *In relation to the number of survived rats with the diagnosis of the first tumor. +In relation to the total number of rats with tumors. $^{x}p<0.05$ compared to AMNU-treated rats.

considerably inhibits chemical carcinogenesis induced by N-nitroso-N-methylurea derivatives.

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