# BIOPHYSICS AND BIOCHEMISTRY

# Effect of β-Carotene on the Plasma Membrane Lipids in Chronically γ-Irradiated Rats

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UDC 616.153.915-092.9-02:615.849.1]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 10, pp. 370-374, October, 1993
Original article submitted May 7, 1993

**Key Words:** *chronic γ-irradiation; membrane; phospholipids; cholesterol; β-carotene*

Chronic γ-irradiation of rats at a dose rate of 12.9 rad/day for 155 days (total dose 20 Gy) does not change the cholesterol level in the brush border membrane (BBM) of the small intestine. The cholesterol content of the BBM increases in rats maintained on a diet enriched with β-carotene (BC) (3 mg/kg body weight throughout the irradiation period). The phospholipid level remains unchanged in rats fed a standard ration and increases in BC-fed rats, the increase being most pronounced for sphingomyelin and phosphatidylserine. The cholesterol/phospholipid ratio was found to be lowered in the BBM of irradiated rats.

As evidenced by 2-$^{14}$C-acetate incorporation, the lipid synthesis in the epitheliocytes of irradiated animals is activated and is not influenced by BC.

The sharp decrease in the cholesterol and phospholipid content of the presynaptic membranes occurring in chronically irradiated rats is normalized by BC. A tendency toward the stimulation of γ-aminobutyric acid (GABA) and L-glutamate transport across the presynaptic membrane of nerve endings and inhibition of L-glutamate transport is revealed in irradiated animals maintained on a BC-enriched ration.

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Thus, a sustained delivery of BC to the body for chronic γ-irradiation has modifying and normalizing effects on plasma membranes.

The mechanisms underlying the molecular and cellular influences of low-dose ionizing irradiation are particularly important in connection with the global contamination of the environment by radionuclides. The requirements for radioprotectors change fundamentally under conditions of chronic irradiation. Such properties as lack of toxicity for chronic administration and ability to elicit both therapeutic and radioprotective effects become indispensable. In our view, naturally occurring metabolism regulators, including β-carotene, are the optimal candidates for a radioprotective agent in chronic irradiation.

The attention of radiobiologists has been drawn to carotinoids due to their involvement in $Ca^{2+}$ metabolism and transmembrane transport [7], the ability to store oxygen in the tissues [3], and the effect on the organism's adaptation to unfavorable environmental conditions [3].

Animal experiments have shown that BC ingested with food is not detected in the blood plasma and is converted to vitamin A in the BBM [10]. The antitumor activity of BC is of great importance: BC-enriched diets reduce the ability of benzpyrene and ultraviolet light to induce skin cancer and lower the susceptibility of mice to carcinoma [13-15].

0007-4888/93/0010-1223$12.50 

TABLE 1. Effect of γ-Irradiation and β-Carotene-Enriched Diet on the Lipid Content in the Brush Border Membrane of the Small Intestine ($M \pm m$)

| Experimental conditions | Ch/Ph | Cholesterol | Sphingomyelin | Phosphatidylcholine | Phosphatidylethanolamine | Phosphatidylserine | Phosphatidylinositides | Phospholipids of total lipid fraction |
|---|---|---|---|---|---|---|---|---|
| Control | 0.38 | 33.4±5.7 ($n$=5) | 19.6±3.1 ($n$=8) | 29.2±9.4 ($n$=8) | 40.7±7.2 ($n$=8) | 17.1±4.9 ($n$=5) | 12.2±3 ($n$=5) | 175.3±33.5 ($n$=6) |
| γ-irradiation (20 Gy) | 0.31 | 38.5±3.8 ($n$=8) | 20.9±1.6 ($n$=8) | 28.3±5.7 ($n$=8) | 43.8±3.8 ($n$=8) | 15.7±1.2 ($n$=5) | 18.7±3.9 ($n$=4) | 250.9±14.5 ($n$=8) |
| γ-irradiation + BC (3 mg/kg/day) | 0.28 | 43.8±5.4 ($n$=5) | 26.9±2.9* ($n$=5) | 50.5±20.3 ($n$=4) | 49.8±10 ($n$=4) | 26.5±6.3 ($n$=3) | 20.6±14 ($n$=3) | 315.4±33.5* ($n$=5) |

Note. Here and in Table 4 an asterisk indicates values statistically significant at $p<0.05$ in comparison with the control; $n$: number of experiments; Ch/Ph: cholesterol/phospholipid molar ratio.

Maintenance of animals under chronic γ-irradiation at a dose rate of 13.5 rad/day during the entire life span cuts longevity by half, predominantly due to the damage to the hemopoietic system and increased incidence of neoplasms [1,6].

It was interesting to examine the lipid metabolism disorders in the plasma membrane of cells exposed for a long time to carcinogenic doses of ionizing radiation and to explore the possibility of modifying or eliminating these disorders by continuous administration of BC.

The object of this study was the plasma membrane of cells resistant to radiation, namely brain neurons and brush border cells of the small intestine. These cells duffer from each other in their longevity: the average life of neurons is comparable to that of the animal, while the brush border cells exist for just 30 h [9].

## MATERIALS AND METHODS

Membranes of brush border cells were prepared as previously [8]. Small intestine epithelial cells were isolated as described [17]. Synaptic membranes were prepared from synaptosomes isolated from rat brain by the method described in [11]. Transport of the neurotransmitter amino acids GABA and L-glutamate across the synaptic membrane was evaluated by the accumulation of $^3$H-GABA and $^3$H-glutamate in incubated synaptosomes.

Isolation and purification of lipids and determination of cholesterol, phospholipid, and protein content were performed as described elsewhere [5]. Phospholipids were chromatographed in a methylacetate:n-propranol-chloroform-methanol-0.25% KCl system (25:25:10:9) [16].

Rats were γ-irradiated in a special cell at a dose rate of 12.9 rad/day for 155 days and received a total dose of 20 Gy. $^{137}$Cs served as the source of γ-radiation.

Throughout the experiment each rat received 3 mg/kg/day BC mixed with cottage cheese. This dose is within the BC concentration range shown to produce an antitumor effect in mice [13,14].

## RESULTS

The cholesterol content of the BBM of irradiated rats was unchanged, while in BC-fed animals this parameter tended to increase. After irradiation the total phospholipid content was slightly increased and the phosphatidylserine content was somewhat decreased. The total phospholipid and sphingomyelin contents were higher in BC-fed animals than in controls. There was a tendency toward a drop of the cholesterol-phospholipid molar ratio after

TABLE 2. Incorporation of 2-$^{14}$C-acetate in the Lipids of the Small Intestine Epithelial Cells in γ-Irradiated and BC-fed rats ($M \pm m$)

| Experimental conditions | Crypt cells | | | Villus cells | | |
|---|---|---|---|---|---|---|
| | total lipids, TA, cpm/mg protein | phospholipids | | total lipids, TA, cpm/mg protein | phospholipids | |
| | | TA, cpm/mg protein | SA, cpm/mg phospholipid | | TA, cpm/mg protein | SA, cpm/mg phospholipid |
| Control | 176±103 | 1897±141 | 1439±388 | 224±91 | 278±144 | 241±120 |
| γ-irradiation (20 Gy) | 398±108 | 678±340 | 728±493 | 650±394 | 671 | 626 |
| γ-irradiation + BC (3 mg/kg/day) | 443±228 | 546±119 | 316±33 | 499±39 | 347 | 375 |

Note. TA: total activity; SA: specific activity.

irradiation, and this was more pronounced in BC-fed irradiated rats (Table 1).

Thus, prolonged γ-irradiation of rats slightly raises the cholesterol and total phospholipid content of the BBM and generates a tendency toward reduction of the phosphatidylserine content. A rise of the BBM phospholipid content was observed in rats irradiated with an equal dose at a high dose rate [8]. Observations of sublethally irradiated rats have revealed sharp drops and rises in the BBM phosphatidylserine content which were particularly apparent during the 1st month after irradiation [12]. A diet enriched in BC increased the phospholipid content of the BBM. The BC-induced increase of the phospholipid content, accompanied by a much smaller rise of the cholesterol content, resulted in a considerable decrease of the cholesterol-phospholipid ratio of the BBM.

There was no statistically significant difference in the cholesterol/phospholipid ratio in irradiated and intact rats, although the ratio was lower in BC-fed animals. It can be suggested that the BC-induced accumulation of the regulatory phospholipids phosphatidylserine and phosphatidylinositide may lower the membrane rigidity, which has a beneficial effect on the irradiated organism.

Since BC affected the phospholipid turnover in the BBM, it was of interest to examine the effect of γ-irradiation on the total lipid and phospholipid synthesis in the cells of the small intestine crypts and villi, as well as on the phospholipid content of epithelial cells.

Generally, total lipid synthesis evaluated by the incorporation of 2-$^{14}$C-acetate determines the *de novo* synthesis of cholesterol and fatty acids (phospholipid precursors) and characterizes the neogenesis of the plasma membrane lipids [4]. It can be seen from Table 2 that lipogenesis in crypt and villus cells of γ-irradiated rats is activated and is not influenced by BC. Increased incorporation of the label in the crypt cell phospholipids may be explained by a high activity of phospholipid acyltransferase. Label incorporation in the phospholipids of the villus cells was much lower. Irradiation decreased the 2-$^{14}$C-acetate incorporation in the phospholipids of crypt cells and increased it in the phospholipids of villus cells. At any rate, γ-irradiation probably influences the lipid differentiation of crypt cells and activates the lipid metabolism in the villus cells. β-Carotene did not affect the label incorporation in phospholipids or the total lipid synthesis in epithelial cells.

Irradiation slightly decreased the phospholipid content of crypt cells and had no effect on the phospholipid content of the villus cells (Table 3).

TABLE 3. Effect of γ-Irradiation and BC-Enriched Diet on Phospholipid Content in Small Intestine Epithelial Cells ($M \pm m$, $n = 3$)

| Experimental conditions | Total phospholipids, mg/mg protein | |
|---|---|---|
| | crypt cells | villus cells |
| Control | 40.5 | 28.7±0.6 |
| γ-irradiation (20 Gy) | 25±2 | 30.8±4.9 |
| γ-irradiation + BC (3 mg/kg/day) | 30.5±5.8 | 36.2±6.2 |

In accordance with the results obtained on the BBM, BC induced a slight increase in the phospholipid content of villus cells in γ-irradiated rats. Consequently, BC facilitates lipid accumulation both in the BBM and in small intestine epithelial cells of irradiated rats.

Our findings indicate that BC exerts no effect on the rate of fatty acid synthesis or on the incorporation of fatty acids in phospholipids.

The metabolic effects of chronic γ-irradiation are probably different from those of acute irradiation (a short-term irradiation with a high dose rate). According to the life duration criterion, a reduction in the dose rate from 200 to 12 rad/day lowers the dose efficacy 10-fold. A 2- to 3-fold increase in the phospholipid content of the BBM was observed 2 h after acute irradiation with a total dose of 20 Gy; a similar increase in the lipid content without any change in lipid synthesis was observed in the villus cells [8]. The metabolic effects of chronic irradiation are unexpectedly high and do not represent a small-scale copy of the effects produced by the same dose of acute irradiation. From the available data we can conclude that ionizing irradiation substantially modifies the metabolism and thus changes the quality of life.

The results obtained in experiments with brain synaptic membranes are indicative of profound metabolic disorders occurring in the brain of chroni-

TABLE 4. Effect of γ-Irradiation and BC-Enriched Diet on Phospholipid and Cholesterol Contents (mg/mg protein) in Presynaptic Membranes Isolated from Rat Brain

| Experimental conditions | Cholesterol | Phospholipids | Cholesterol/phospholipid molar ratio |
|---|---|---|---|
| Control | 73±20 | 698±10 | 0.2 |
| γ-irradiation (20 Gy) | 48±3 | 436±34* $p<0.001$ | 0.22 |
| γ-irradiation + BC (3 mg/kg/day) | 88±1 | 774±32 | 0.23 |

TABLE 5. Transport of Neurotransmitter Amino Acids in Synaptosomes Isolated from the Brain of Control and Chronically Irradiated Rats and BC-Fed Irradiated Rats ($M \pm m$, $n=3$)

| Experimental conditions | Accumulation of neurotransmitter amino acids in synaptosomes | | | |
|---|---|---|---|---|
| | $^3$H-glutamate, nmol/mg protein/10 min | % of control | $^3$H-GABA, nmol/mg/protein/10 min | % of control |
| Control | $(1.519 \pm 0.23) \times 10^{-9}$ | 100±15 | $(1.186 \pm 0.17) \times 10^{-9}$ | 100±14 |
| Chronic γ-irradiation (20 Gy, 155 days) | $(1.692 \pm 0.29) \times 10^{-9}$ | 112±19 | $(1.309 \pm 0.25) \times 10^{-9}$ | 110±21 |
| Chronic γ-irradiation + BC (5 mg/kg/day) | $(1.148 \pm 0.045) \times 10^{-9}$ | 76±4 | $(1.321 \pm 0.056) \times 10^{-9}$ | 111±5 |

cally irradiated rats. Synaptic membranes of the brain neurons have almost twice the amount of cholesterol per mg protein as compared with the BBM. After chronic irradiation this parameter was dramatically decreased (Table 4) and normalized in BC-fed rats.

The phospholipid content of synaptic membranes is much higher than that of the BBM, and therefore the cholesterol/phospholipid molar ratio is lower in synaptic membranes than in the BBM.

The phospholipid content was markedly decreased in irradiated rats and reached the original level in BC-fed rats.

The value of the cholesterol/phospholipid ratio remained unchanged.

It is known that the modification of lipids in the synaptic membrane of nerve endings by phospholipids, phospholipases, unsaturated fatty acids, and antioxidants alters the functional activity of the transport proteins which are involved in reverse uptake of neurotransmitters from the synaptic cleft to the nerve endings [2]. We revealed a tendency toward stimulation of the transport of the neurotransmitter amino acids $^3$H-L-glutamate and $^3$H-GABA in synaptosomes isolated from the brain of chronically irradiated rats (Table 5). In BC-fed rats GABA transport remained unchanged, while glutamate transport was lower than in the controls.

In irradiated rats the restoration of the synaptic membrane lipid components (evaluated by cholesterol and total phospholipid contents) was accompanied by modification of the functional activity of transport proteins involved in the reverse uptake of the excitatory neurotransmitter L-glutamate.

The results obtained are of special interest. The ability of the metabolism regulators to modify the damage of γ-irradiation can be utilized in the primary screening of radioprotective preparations. We observed no profound metabolic disturbances in the BBM of chronically irradiated rats.

The metabolism of the plasma membranes of neurons, cells which have a long life span and are resistant to irradiation, is much more sensitive to chronic irradiation than the metabolism of the villus cells, which have a short life span. β-Carotene completely normalized the metabolic effect of chronic γ-irradiation on the lipid composition of the synaptic membranes of nerve endings. Accumulation of phospholipids, particularly of sphingomyelin, in the brush border of the small intestine epithelial cells can be regarded as a process improving the condition of an irradiated animal.

The data on the high sensitivity of lipid metabolism of the neuronal neurotransmitter systems to chronic γ-irradiation at a low dose rate may be useful for the formulation of concepts regarding the mechanisms underlying the damaging effect of chronic irradiation.

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