

EFFECT OF ASCORBIC ACID ON CARCINOGENICITY
OF p-HYDROXYPHENYL-LACTIC ACID

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Interest in ascorbic acid as a supplementary agent with a positive effect in the treatment of cancer has increased considerably in the last decade. Ascorbic acid has been shown to have a distinct inhibitory effect on carcinogenesis induced in experimental animals by precursors of nitrosamines [7], by preventing the formation of carcinogens from them by metabolites of tryptophan and tyrosine [3, 8], an effect primarily linked with the properties of ascorbic acid as an active antioxidant [6], or explained by the specific participation of this vitamin in the metabolism of these amino acids.

The study of the anticarcinogenic activity of ascorbic acid in carcinogenesis induced by tyrosine metabolites is particularly interesting because it is now known that tyrosine metabolism is disturbed in patients with various types of leukemia and, as a result, a high level of tyrosine breakdown products — p-hydroxyphenyl-lactic and p-hydroxyphenylpyruvic acids (PHPLA and PHPPA respectively), is observed in the urine and blood [4, 5]. One of these products, namely PHPLA, exhibits high carcinogenic activity in experiments on mice [1], and a preliminary investigation has shown that this activity is inhibited by ascorbic acid [3].

The present investigation, conducted on an extensive material, is a continuation of a study of the effect of ascorbic acid on the carcinogenic activity of PHPLA.

EXPERIMENTAL METHOD

Altogether 100 C57BL mice (50 females and 50 males), taking part in the experiment at the age of 2 months, were under observation. The animals were divided into two equal groups and vaccinated against mouse pox with vaccinia virus. Mice of group 1 received PHPLA alone, mice of group 2 received PHPLA accompanied by ascorbic acid.

PHPLA was injected subcutaneously for 5 weeks in a dose of 5 mg twice a week. Ascorbic acid was given to the animals with the drinking water in a dose of 250 mg to 100 ml water [8], parallel with administration of PHPLA, and later throughout the period of observation. The mice were inspected periodically and blood tests were carried out until the age of 22-24 months, after which they were sacrificed. Dying and killed animals were autopsied. The lymph nodes, lungs, thymus, liver, kidneys, spleen, urinary bladder, submaxillary salivary gland, sternum, femur, and ribs were taken for histological analysis. Organs and tissues were fixed in 10% formalin solution and embedded in paraffin wax, and sections were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Analysis of the results shows (Table 1) that injection of PHPLA into the mice of group 1 led to induction of tumors in 55% of the animals studied. Meanwhile injection of PHPLA with ascorbic acid caused a marked decrease in the carcinogenicity of PHPLA (22.5% of tumors). The differences found in the frequency of induction of neoplasms in the two groups of animals

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TABLE 1. Effect of Ascorbic Acid on Carcinogenicity of PHPLA

Group of animals	Number of animals	Time of appearance of first tumor, months	No. of animals surviving until appearance of first tumor	Number of animals with neoplasms	Frequency of neoplasms, %	Type of neoplasm										
						hemoblastosis	papilloma of urinary bladder	neptoma	hemangioendothelioma of liver	adenoma of lungs	adenoma of pancreas	cystic epithelioma of skin	osteoma	chondroma	leiomyosarcoma	papilloma of renal pelvis
1- (PHPLA)	50	16,2	42	23	55	19	2	—	1	—	1	1	1	1	1	—
2- (PHPLA + ascorbic acid)	50	16,2	40	9	22,5	8	—	1	—	1	—	—	—	—	—	1

are highly statistically significant ($P < 0.01$).

Morphological analysis showed that the neoplasms most frequently found in the experimental animals were hemoblastoses — in 19 and eight cases respectively in animals of groups 1 and 3 ($P < 0.01$). The overwhelming majority of cases of this form of neoplasia were accounted for by lymphosarcomas of mixed type, diagnosed mainly in mice aged 22–24 months, and unaccompanied in their course by any marked hematologic changes. As a rule at autopsy on these animals growths of the internal organs were observed, affecting most frequently the thymus, spleen, and mesenteric lymph nodes. Characteristically other forms of neoplasms diagnosed in the two groups of animals (Table 1) differed significantly in their morphological characteristics. Most tumors in the group of animals not receiving ascorbic acid appeared much sooner (before the age of 90 weeks) than in the animals of group 2. In addition, in the animals of group 1 precancers were observed in three cases and hyperplasia of the transitional epithelium of the urinary bladder in nine cases, whereas in the animals of group 2, receiving ascorbic acid, these forms of precancer were not discovered and the frequency of focal hyperplasia of the epithelium of the urinary bladder only one-third as high.

When the results of this investigation are assessed it will be noted that the data on the carcinogenic activity of PHPLA (55%) are almost exactly the same as the results of the similar investigation published previously (60%) [1, 2]. There is likewise no doubt about the tendency of the carcinogenic effect of PHPLA to be directed toward leukemia.

Comparison of the frequency of discovery and the morphological characteristics of neoplasms diagnosed in the animals points to the existence of a distinct inhibitory action of ascorbic acid on the carcinogenic activity of PHPLA, confirming the results of previous investigations [3]. First and foremost there is a reduction by half in the number of neoplasms in the group of mice receiving PHPLA together with ascorbic acid. The addition of ascorbic acid to the diet of these animals appreciably altered the morphological characteristics and delayed the time of appearance of the neoplasms, making these parameters very similar to the data on spontaneous neoplasms in C57BL mice [2]. Meanwhile neoplasms arising in the animals of group 1 were found exceptionally rarely among spontaneous tumors in mice of this line, and they must therefore have evidently been induced. The appearance of a fairly high percentage of neoplasms diagnosed in the animals of group 2 (22.5%), somewhat higher than their number in healthy animals (14%) [2] must be noted, and it could be the result of the use of insufficient vitamin C loads, so that further modifications to the doses and times of its administration are necessary.

Other evidence in support of the inhibitory effect of ascorbic acid on carcinogenesis, in the writers' opinion, is the considerable fall in the frequency of discovery of precancerous changes.

It can accordingly be concluded from these results that ascorbic acid has a marked inhibitory action on carcinogenesis induced by PHPLA in C57BL mice.

LITERATURE CITED

1. E. I. Zharova, T. I. Sergeeva, V. D. Ivanova, et al., *Probl. Gematol.*, No. 6, 34 (1974).
2. E. I. Zharova and T. I. Sergeeva, in: *The Role of Endogenous Factors in the Development of Leukemias* [in Russian], Moscow (1974), p. 34.
3. E. I. Zharova and T. I. Sergeeva, in: *Mechanisms of Anticarcinogenesis* [in Russian], Kiev (1974), p. 46.

4. V. D. Ivanova, in: Role of Endogenous Factors in the Development of Leukemias [in Russian], Moscow (1974), p. 9.
5. V. D. Ivanova, V. N. Baikova, M. M. Kaverzneva, et al., Probl. Gematol., No. 4, 25 (1979).
6. E. Cameron, L. Pauling, and B. Leibovitz, Cancer Res., 39, 663 (1979).
7. S. S. Murvish, Ann. N.Y. Acad. Sci., 258, 432 (1975).
8. G. E. Pipkin, I. U. Shlegel, R. Nishimura, et al., Proc. Soc. Exp. Biol. (N.Y.), 131, 151 (1969).

EFFECT OF APPENDECTOMY ON THE ANTITUMOR EFFECT OF ALL-TRANS-METHYLRETINOATE

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Data have now been obtained on the role of the lymphoid tissue of the appendix in immune defense [4, 11, 13]. There is limited information on the results of statistical estimations of the degree of correlation between a previous appendectomy and the risk of malignant disease in man [5, 8, 9]. According to McVay [10], appendectomy was present in the history of 18.3% of persons dying from carcinoma of the large intestine and 12.6% dying from carcinoma elsewhere.

Vitamin A and its synthetic analogs, the retinoids, have the property of inhibiting growth of tumors mainly of epithelial nature and they have a protective action on epithelium against carcinogens. These compounds also have the property of inducing nonspecific stimulation of the immunocompetent system [3].

The aim of the present investigation was to study changes in the antitumor properties of the retinoid all-trans-methylretinoate (MR) after removal of the appendix, as an organ belonging to the immunocompetent system.

EXPERIMENTAL METHOD

Experiments were carried out on 70 adult mature Wistar rats whose appendix was removed under ether anesthesia. For this purpose an incision was made in the skin and abdominal wall measuring 2-2.5 cm in length on the left side of the midline. The appendix was removed and the stump ligated. The wound was closed in layers. Control animals underwent a laparotomy at the identical spot, followed by suture of the abdominal wall and skin. A carcinoma RS-1 was inoculated subcutaneously into the animals 15-18 days after the operation. The tumor was injected as a cell suspension in medium No. 199 in the ratio of 1:3 and in a dose of 0.5 ml. The day of inoculation of the tumor was counted as the beginning of the experiment. Changes in the dynamics of tumor growth were judged by reference to the carcinosomatic index (CSI), determined as the ratio (in %) between the weight of the tumor and the animal's body weight together with the tumor on the day of sacrifice.

A 1% oily solution of MR was used as the retinoid (the preparation was obtained from the Laboratory of Chemistry of Polyene Compounds, "Vitaminy" Scientific Production Combine, Ministry of the Oil Industry of the USSR), which was injected intraperitoneally in a dose of 0.5 ml once a week for 1 month. The effect of appendectomy on the CSI of carcinoma RS-1 was studied in a control experiment lasting 23 days. The animals were given an intraperitoneal injection of 2 ml of 50% colloidal carbon 2 h before sacrifice. Immediately before sacrifice

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