ANTITUMOR AND TOXIC PROPERTIES OF RETINOID C_{15}

Yu. I. Afanas'ev, V. I. Nozdrin,
M. Z. Bakhshinyan, G. I. Samokhvalov,
L. A. Vakulova, and I. M. Yakovleva

UDC 615.356:577.161.1].017:615.277.3+615.356:577.161.1].099

KEY WORDS: vitamin A; toxicity; retinoic acid; metabolites.

One factor which limits the experimental and clinical use of vitamin A is its toxicity. The spectrum of action of vitamin A metabolites is narrower, and it is therefore possible that by using these metabolites the desired effect may be achieved with less marked toxicity. For instance, whereas retinyl palmitate activates humoral and cellular immune responses, retinoic acid, which is evidently the closest metabolite of vitamin A [2], stimulates mainly the production and functional activity of T killer lymphocytes [5]; at the same time, the antitumor effect of retinoic acid is also accompanied by less marked toxicity [1]. It can accordingly be postulated that not only retinoic acid itself, but also its metabolites, participate in the mechanism of the adjuvant effect.

This paper gives the results of a study of the antitumor and toxic properties of retinoid C_{15}, a synthetic analog of natural vitamin A metabolites.

EXPERIMENTAL METHOD

Retinoid C_{15} diester (ethylester of 2E,4E-3-methyl-5/2,6-dimethyl-6-ethoxycarbonyl-3-oxo-1-cyclohexen-1-yl-2,4-pentadien-1-ic acid) was synthesized by the method described previously [3, 4]. Retinoid C_{15}-diacid was obtained by gentle oxidation of the trans isomer of the C_{15} diester. Experiments were carried out on adult sexually mature CBA, C57BL/6, and (C57BL × CBA)F_1 mice of both sexes and on noninbred animals, into which the following tumors were inoculated subcutaneously and intramuscularly in medium 199 in the ratio of 1:3 — carcinoma of the cervix uteri (CCU5), carcinoma of the forestomach (CFS), and Lewis carcinoma (lung tumor), Retinoid C_{15} was injected intraperitoneally in the form of oily and alcoholic solutions on alternate days in a dose of 0.2-1.6 mg. The acute toxicity of the compounds was estimated from the maximal tolerated dose (MTD) and the dose causing death of half of the animals used in the experiments (LD_{50}), which were determined as described previously [1]. Chronic toxicity was judged from the ability of the animals to survive until the end of the experiment, changes in their weight, and also changes in the hemoglobin concentration and erythrocyte and leukocyte counts in their blood, and changes in the number of functional activity of the macrophages in the liver, spleen, and lymph nodes, and from morphological parameters: the area of the lymphoid follicles of the spleen and the relative areas of the paracortical zone, lymphoid follicles, and medullary cords of a mesenteric lymph node. To label the macrophages, 2 h before sacrifice the animals received an intraperitoneal injection of 0.5 ml of 50% colloidal carbon. Before sacrifice, blood was taken from the caudal veins; the hemoglobin concentration and erythrocyte and leukocyte counts were determined by the usual methods. The animals were killed with ethyl ether. Material was fixed in a mixture of 40% formaldehyde, 96% ethyl alcohol, and glacial acetic acid in the ratio of 9:3:1. The number of cells taking up colloidal carbon was counted in sections of the liver and spleen. Phagocytic activity was determined by counting the mean number of granules of carbon per macrophage, and also the mean number of cells with superintensive phagocytosis, in which the number of granules could not be counted because they were too numerous. Antitumor activity was judged by the ratio of the weight of the tumor to the body weight of the animal.

Department of Histology and Embryology, I. M. Sechenov First Moscow Medical Institute.
(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovalev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 76-78, April, 1982. Original article submitted March 3, 1981.

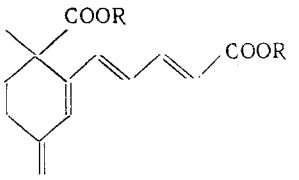
0007-4888/82/9304-0479$07.50

TABLE 1. Effect of Intraperitoneal Injection of Retinoid C_{15}-Diester on Survival Rate, Body Weight, and Tumor Weight in Mice (10th day of experiment, total dose of retinoid for each animal 0.005 g)

Line of animals	Type of tumor	Treatment	Survival rate	Body weight, g	Tumor weight, g	Ratio of tumor weight to body weight, %
C57Bl/6	Lewis carcinoma	Retinoid C_{15}	10/10 (100%)	21,7±0,3	2,7±0,01	12,4
		Methyl retinoate	11/10 (91%)	21,1±0,06	2,0±0,01	9,5
		Solvent	10/9 (90%)	21,1±0,3	3,4±0,01	16,1
		Control (intact animals)	10/10 (100%)	21,5±0,5	—	—
CBA	CFS	Retinoid C_{15}	11/10 (91%)	29,3±1,9	1,6±0,08	5,5
		Methyl retinoate	11/9 (82%)	24,8±1,3	1,9±0,07	7,7
		Solvent	9/8 (88%)	26,2±1,5	2,3±0,1	8,8
		Control (intact animals)	15/15 (100%)	33,3±0,3	—	—
C57Bl/6	CCU5	Retinoid C_{15}	11/10 (91%)	24,9±0,2	1,7±0,02	6,8
		Methyl retinoate	11/9 (82%)	19,7±0,04	1,1±0,01	5,6
		Solvent	10/10 (100%)	22,3±0,2	1,9±0,04	8,5
		Control (intact animals)	15/15 (100%)	23,4±0,2	—	—

EXPERIMENTAL RESULTS

Retinoid C_{15} had the following structure:



i.e., it could exist in either ester (R-alkyl) or acid (R = H) forms.

The substance had certain characteristic features of the structure of natural vitamin A metabolites and of retinoic acid, especially a strongly oxidized β-ionic ring and a shortened isoprene chain.

This paper gives mainly the results of biological tests using retinoid C_{15}-diester for in this form the substance was only one-third as toxic.

Investigation of the antitumor properties of the compound showed that retinoid C_{15} can delay growth of primary tumors. For instance, the weight of a Lewis carcinoma, which grows more rapidly than the other tumors, reached 3.4 ± 0.01 g toward the end of the experiment. Delay of growth of the tumor on the 10th day of the experiment, caused by injection of retinoid C_{15} in a dose of 0.001 g on alternate days, was not significant. The ratio of tumor weight to body weight for the mice of the control group was 16.1%, and for those of the experimental group 12.4% ($P < 0.05$). The CFS grew more slowly and by the 11th day of the experiment its weight was 8.8% of the body weight. The compound, injected in a dose of 0.001 g on alternate days, reduced this ratio to 5.5% ($P > 0.05$). In animals with an inoculated slowly growing CCU5 tumor, the compound administered for 30 days in the same dose as in the previous experiments lowered the tumor weight in body weight ratio from 8.5 to 6.8% ($P = 0.05$; Table 1). The results show that the antitumor effect of retinoid C_{15} depends on the determinable degree of malignancy of the tumor as reflected in the time course of its growth. Retinoid C_{15} was less toxic than retinoic acid. In acute experiments MTD for retinoic acid was 0.3 g/kg and for retinoid C_{15}-diester 1.5 g/kg; LD_{50} for retinoic acid was 216 mg/kg and for retinoid C_{15} 1058 mg/kg. In chronic experiments animals receiving retinoic C_{15}-diester had a rather higher survival rate and body weight than animals receiving methyl retinoate (Tables 1 and 2). Like methyl retinoate, retinoid C_{15} reduced the erythrocyte count and hemoglobin concentration in the blood (Table 2), evidently because of the ability of increased doses of vitamin A and retinoids to damage the erythrocyte membrane [6]. However, in the case of retinoid C_{15} this property was weaker. Intraperitoneal injection of two retinoids was accompanied by peritonitis, and this was reflected evidently in these animals by the high blood leukocyte count. Retinoid C_{15} had more marked inflammatory properties (Table 1). Injection of the substance was accompanied by a moderate decrease in the number and phagocytic activity of the macrophages

TABLE 2. Effect of Intraperitoneal Injection of Retinoid C_{15}-Diester on Hemoglobin Concentration (HB), Erythrocyte Count (RBC), and Leukocyte Count (WBC) in Female CBA Mice Inoculated with CFS Tumor and of C57BL/6 Mice Inoculated with CCU5 Tumor

Type of tumor	Treatment	Hb, g%	RBCs in 1 mm^3	WBCs in 1 mm^3
CFS	Retinoid C_{15}	11,7±0,6	4 100 000±30 000	31 580±280
	Methyl retinoate	10,8±0.08	3 920 000±14 000	17 060±410
	Solvent	14,8±0,08	5 560 000±21 000	11 500±850
CCU5	Control (intact CBA mice)	15,6±0,07	6 510 000±10 000	3 400±250
	Retinoid C_{15}	12,1±0,2	4 620 000±350 000	4 560±720
	Methyl retinoate	11,8±0,1	4 650 000±320 000	35 550±260
	Solvent	14,9±0,07	5 950 000±360 000	10 450±380
	Control (intact C57Bl/6 mice)	15,4±0,08	6 870 000±40 000	8 400±290

TABLE 3. Effect of Intraperitoneal Injection of Retinoid C_{15}-Diester on Number and Phagocytic Intensity of Macrophages in Liver and Spleen [male (C57BL × CBA)F_1 mice, 12th day of experiment, magnification 900 ×]

Type of tumor	Treatment	No. of macrophages		Phagocytic intensity of macrophages		No. of macrophages with superintensive phagocytosis	
		in liver	in spleen	in liver	in spleen	in liver	in spleen
Lewis carcinoma	Retinoid C_{15}	6,2±0,2	2,1±0,04	14,6±0,6	8,5±0,6	0,2±0,05	0,1±0,03
Lewis carcinoma	Solvent	6,3±0,1	3,7±0,1	19,2±0,6	10,1±0,3	0,5±0,02	0,2±0,02
Control (intact animals)	—	6,0±0,1	2,6±0,004	17,7±0,5	8,3±0,6	0,4±0,06	0,2±0,03

TABLE 4. Effect of Intraperitoneal Injection of Retinoid C_{15}-Diester on Number per Field of Vision of Light Microscope (magnification 160 ×) and Area of Lymphoid Follicles (in conventional units) in Spleen and Ratio between Areas of Zones (in conventional units) of Lymphoid Tissue of Mesenteric Lymph Node [(C57BL × CBA)F_1 mice; 12th day of experiment]

Type of tumor	Treatment	Lymphoid follicles in spleen		Lymphoid tissue of lymph node		
		number	urea	PCZ, %	LF, %	MC, %
Lewis carcinoma	Retinoid C_{15}	3,5±0,6	10,6±1,0	58,7	11,4	27,0
Lewis carcinoma	Solvent	1,7±0,3	12,6±1,1	48,5	8,8	42,7
Control (intact animals)		1,4±0,3	12,0±1,2	37,1	11,8	51,1

in the liver and spleen (Table 3), which evidently reflected the ability of these cells to store and metabolize retinoids in the peritoneal cavity as the site of their highest concentration [7].

Retinoid C_{15} not only did not depress lymphocytopoiesis in the lymphoid organs, but either increased the size of the lymphocyte population or changed the ratio between their subpopulations. For instance, administration of the compound was followed by an increase in the area of the paracortical zone of the mesenteric lymph node and an increase in the number of small lymphoid follicles without any clearly defined reactive centers in the spleen (Table 4).

The data show that the synthetic retinoid may be interesting for further study of its effect on the origin and development of tumors, for this substance is less toxic, it can inhibit tumor growth, but it does not inhibit the immune system.

Retinoid C_{15} containing an oxidized β-ionic ring and a shortened isoprene chain was thus synthesized. The substance inhibited growth of primary tumors; this property depended on the rates of growth of the tumors. The compound had lower acute and chronic toxicity than retinoic

acid and methyl retinoate. Administration of the compound was accompanied by widening of the paracortical zone of the mesenteric lymph node and by an increase in the number of lymphoid follicles in the spleen.

The authors are grateful to Senior Laboratory Assistant V. M. Padalko of the Department of Histology and Embryology, I. M. Sechenov First Moscow Medical Institute, for help with the blood investigation.

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CHANGES IN THE CHARACTER OF HEPATOCYTE PROLIFERATION IN THE LIVER AND ADENOMATOUS NODES IN MICE DURING CCl_4-INDUCED CARCINOGENESIS

V. M. Faktor, V. S. Poltoranina, and I. V. Uryvaeva

UDC 616.36-006-092.9-02:615.277.4:547.412.133-07:616.36-018.15

KEY WORDS: hepatocytes; liver; adenomatous nodes; carcinogenesis.

Ontogenetic and reparative growth of the rat and mouse liver is characterized by the progressive development of cell polyploidy, as a result of a process of mitotic polyploidization that is typical of hepatocytes [1]. Meanwhile reports of the accumulation of cells with a low level of ploidy in the early stages of neoplastic growth [10] and of the diploid composition of hepatomas [6, 7, 9, 11, 12] have been published.

Nodular proliferation of hepatocytes is a relatively early reaction of the liver to carcinogens. The study of the cell composition of the nodules can provide information on the sources of abnormal growth and on the characteristics of cell division. In the present investigation the DNA content was studied in hepatocytes during the development of nodular proliferation and also separately in cells isolated from large adenomatous nodes and the surrounding liver. The character of the cell transformations in the course of division was judged by measuring the DNA content in undividing cells, cells synthesizing DNA, and postmitotic cells by a combination of cytospectrophotometric and autoradiographic methods with double isotope labeling.

EXPERIMENTAL METHOD

An oily solution of CCl_4 was given perorally to SWR mice in a dose of 0.1 ml twice a week [8]. The animals' liver was studied after 1, 7, 41, and 52 doses of CCl_4, in the last case 4 months after the end of its administration. Two days after the single or last dose of CCl_4 (except in the case of 52 doses), [^{14}C]thimidine was injected intraperitoneally into

Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR. Laboratory of Immunochemistry and Diagnosis of Tumors, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 79-82, April, 1982. Original article submitted November 18, 1981.

 0007-4888/82/9304-0482$07.50