EFFECT OF LIVER RNA ON THE GROWTH OF TUMORS IN EXPERIMENTAL ANIMALS

(UDC 616-006-085.739.6-092.9)

K. A. Perevoshchikova, A. P. Belousov, and T. V. Bul'dyaeva

Biochemical Laboratory (Head—Doctor of Biological Science, A. P. Belousov),
P. A. Gertsen Oncological Institute (Director—Doctor of Medical Sciences,
Professor A. N. Novikov), Moscow
(Presented by Member of the Academy of Medical Sciences, USSR, A. I. Savitskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 8,
pp. 102-105, August, 1965
Original article submitted January 10, 1964

The available literature data [1, 3, 5, 6] note the inhibiting effect of RNA from normal tissues on the growth of transplanted animal tumors. In particular, it has been shown [1] that RNA from liver cells inhibits the growth of mucous cancer of this organ in rats.

In this work we tested the effect of RNA isolated from the rat liver on the growth of certain transplanted tumors in experimental animals.

EXPERIMENTAL PROCEDURE

RNA from the rat and mouse liver was isolated by Kirby's phenol method in the modification of G. P. Georgiev [2] and V. I. Vorob'ev [1], and by Scherrer's method [8].

As a rule, traces of protein (0.12-0.015%) were detected in the RNA preparation; there was no DNA (negative reaction with diphenylamine) and glycogen. The RNA obtained was placed in physiological saline (3-4 mg per ml) and mixed in a 5:1 ratio with a suspension of cells of M-1 sarcoma or with cells of Ehrlich's ascitic carcinoma of mice and ascitic hepatoma of rats, washed with this solution. In the control, an equal amount of physiological saline was added to the suspension of cells.

After the addition of RNA (final concentration 2.5-3 mg per ml), the mixture was incubated for 4 h at room temperature, and in a number of experiments for 18 h at 4° in a refrigerator. After incubation, the suspension of tumoral cells was injected subcutaneously (in the case of M-1 sarcoma) and intraperitoneally (in the case of the ascitic form of carcinoma) in 1 ml doses. Cytological testing of the cells of Ehrlich's ascitic carcinoma after their incubation with liver RNA showed that they were morphologically undamaged.

The animals were killed 7-10 days after the transplant. The tumors of the animals of the experimental and control groups were weighed, and the percent inhibition of tumor growth was calculated according to the formula:

$$\frac{M_к - M_о}{M_к} \times 100,$$

where $M_к$ is the average weight of the tumor in the control group, $M_о$ is the average weight of the tumor in the experimental group [1].

At the same time, an in vitro investigation was made of the effect of liver RNA preparations on the incorporation of labeled amino acids into the proteins of cells of Ehrlich's ascitic carcinoma from mice.

EXPERIMENTAL RESULTS

First of all, we tested the effect of RNA produced from mouse liver by Kirby's phenol method in the modification of G. P. Georgiev on the growth of Ehrlich's ascitic carcinoma in these animals. In 4 series of experiments (30

TABLE 1. Effect of Liver RNA from Normal Rats on the Growth of M-1 Sarcoma

Type of tumor	Method of isolating RNA from rat liver	No. of animals used in the series of expt.	Average wt. of tumors of control animals (in g)	Average wt. of tumors of experimental animals (in g)	Inhibition of tumor growth (in %)
M-1 sarcoma	According to Kirby method in modification of	20	11.5	4.8	58.0
	V. I. Vorob'iev	20	18.5	7.9	57.3
	The same	10	1.4	0.1	93.0
	According to Scherrer method	20	21.8	2.5	88.5
	The same	20	13.5	7.4	45.1
	According to Kirby method in modification of	20	6.2	1.1	81.0
	G. P. Georgiev	20	20.0	19.5	2.5
	The same	20	19.6	18.0	8.0
	" "	20	17.0	163.	4.0
	" "	20	25.0	23.0	8.0

mice in each), no differences were detected in the rate of growth of the tumors in the animals of the experimental and control groups. The RNA preparation obtained from rat liver was also tested for survival of cells of M-1 sarcoma of rats, preliminarily incubated with normal liver RNA. The results obtained were negative (Table 1). However, in the case of ascitic hepatoma of rats, a certain tendency toward deceleration of rejection of the tumors in the animals of the experimental group was observed in a number of experiments.

Since an inhibiting effect of liver RNA on the growth of rat cholangioma has been detected [1], we repeated these experiments. In this case we used RNA preparations isolated by the phenol method in V. I. Vorob'iev's modification. In 10 rats that received subcutaneous injections of a suspension of tumoral cells, treated with liver RNA, no tumor appeared, at the same time, tumors were observed in 100% of the cases in the control group.

In view of this, in our further work we produced RNA chiefly by the phenol method in V. I. Vorob'iev's modification.

The effect of RNA on the growth of rat M-1 sarcoma was tested. As can be seen from Table 1, in 2 series of experiments inhibition of tumor growth was observed (by 57 and 57.3%), while in 2 other series, the inhibition was even more pronounced (93 and 88.5%). And yet, externally the tumors in the animals of the experimental groups did not differ from those in the rats of the control group (no morphological investigations were conducted), and there was no 100% rejection of the tumor, as noted in experiments in the case of cholangioma.

Cytoplasmic RNA and RNA of the nuclear fluid can be isolated by the phenol method in V. I. Vorob'iev's modification [1]. These RNA fractions evidently are the active ingredients. According to the recently described phenol method of Scherrer [8], the total cellular RNA (including m-RNA) is extracted in the modification that he proposes.

We attempted to isolate RNA from the liver and rat M-1 sarcoma by this method. It was found that the inoculation of tumoral cells preliminarily incubated with liver RNA was delayed in comparison with the control. Growth of the tumor was 81% inhibited in one case, and 45.1% inhibited in another. If the tumoral cells were incubated with RNA from M-1 sarcoma, produced by the same method, then the rate of tumor growth in the experimental group of animals lagged behind, but not in all the series of experiments, and to a lesser degree.

A series of investigations was conducted on rat ascitic hepatoma (280 animals). The tumoral cells were incubated both with RNA produced by the Kirby phenol method in V. I. Vorob'iev's modification, and with RNA isolated according to Scherrer. In both cases, both positive and negative experimental results were obtained.

In the first case, in 2 experiments (10 animals in each), no ascitis appeared, in contrast to 100% successful grafting in the control group, while in the other 2 experiments, tumors appeared both in the control and in the experimental groups.

TABLE 2. Incorporation of Labeled Amino Acids into Cells of Ehrlich's Ascitic Carcinoma of Mice After their Preliminary Incubation with Normal Liver RNA of Mice in Vitro

Index	Incorporation of labeled amino acids (in counts/min/10 mg)												
	glycine-C^{14}				aver. of 4 expts.	leucine-C^{14}							aver. of 7 expts.
	experiment					experiment							
	1	2	3	4			2	3	4		6	7	
Inhibition in experimental group (in % of control I)*	49.1	60.5	53.1	68.8	57.8	25.6	66.2	39.3	69.4	38.2	55.5	47.0	48.7
Inhibition in control II† (in % of control I)	–	–	–	–	–	–	75.7	19.0	–	47.0	54.9	–	49.1

* Experimental: cancer cells were incubated with liver RNA. Control I: cancer cells were incubated with physiological saline.

† Control II: cancer cells were incubated with tumor RNA.

In the 2nd case (RNA isolated according to Scherrer), in 3 experiments there were no tumors in the animals of the experimental groups, while in 7 experiments, ascitic hepatoma developed in the animals both of the experimental and of the control groups (20 rats in each experiment).

Thus, in the case of ascitic hepatoma in rats, we were unable to obtain unambiguous results. We shall devote our further investigations to determining the cause of this.

In addition to studying the biological properties of liver RNA, we attempted to determine the effect of the preparations obtained on protein synthesis of cells of Ehrlich's ascitic carcinoma of mice in vitro. For this purpose, the tumor cells, preliminarily treated with RNA from normal liver cells, were incubated for 50 min in Robinson's medium [7] at 37.8° with leucine-C^{14} or with glycine-C^{14}. It was shown (Table 2) that the incorporation of glycine-C^{14} into the proteins of tumor cells treated with RNA is sharply reduced: it fluctuates in various experiments from 49.1 to 68.8% (57.8% on the average). In the case of leucine, the range of fluctuations is broader (from 25 to 69.4%, average 48.7%). However, in all cases the tumoral cells that were preliminarily incubated with liver RNA incorporated amino acids into their proteins at a lower rate in comparison with the control. The incorporation of leucine-C^{14} into the proteins of tumoral cells that were preliminarily incubated with a preparation of tumor RNA was also reduced (49.1% inhibition).

Consequently, in this case the effect of liver RNA on protein biosynthesis in tumoral cells is nonspecific.

The inhibiting effect of RNA of normal tissues on the development of a number of tumors has now been revealed. It has been found that certain tumors are insensitive to the action of RNA; in a number of cases, not only liver RNA, but also RNA obtained from tumoral tissue slows down the growth of tumors in animals [1, 4]. Thus, French researchers [4], in contrast to other authors [5, 6], observed a substantial inhibition of tumor growth (FLS ascitic tumor) after treatment of tumoral cells with liver RNA only when the RNA dose was equal to 10 mg per 10^7 cells. In this case, the difference between the effect of liver RNA and RNA from tumoral cells was not so great as was shown in the experiments of Niu [5, 6].

In our experiments, the inhibiting effect of normal liver RNA was noted only in the case of M-1 sarcoma, and this effect is evidently nonspecific, since in a number of experiments, a reduction of the tumor growth was also observed when the tumoral cells were incubated with tumor RNA. The nonspecificity of the effect of RNA is also confirmed by the data of experiments in vitro; inhibition of protein synthesis occurred here when not only liver RNA, but also tumor RNA was added to the incubation medium.

SUMMARY

A study was made on the effect of RNA recovered from the rat liver by various methods on the growth of transplantable tumors in experimental animals. It was shown that RNA from the liver cells produced by Kirby's phenol method in V. I. Vorobyov's modification or after Scherrer inhibited the growth of sarcoma M-1 in rats in 45-93% of cases. In case of ascitic hepatoma of rats the inhibiting effect of RNA is not always apparent. The tumor-inhibiting effect of the liver RNA depends, to a certain extent, on the method used for RNA recovery. The effect of RNA on the growth of sarcoma M-1 and protein synthesis in tumor cells (in vitro) is apparently not specific.

1. N. N. Aksenova, V. M. Bresler, and V. I. Vorob'ev et al., Tsitologiya, 5 (1962), p. 490.
2. G. P. Georgiev, Biokhimiya, 3 (1959), p. 472.
3. A. K. Griffin and M. A. O'Neil, Summaries of Reports at the Eighth International Anticancer Congress [Russian translation] Moscow (1962), p. 100.
4. J. Harel, F. Lacour, and C. Verger, C. R. Acad. Sci. (Paris) 254 (1962), p. 4390.
5. M. C. Niu, Science, 131 (1960), p. 1321.
6. M. C. Niu, C. C. Cardova, and L. C. Niu, Proc. nat. Acad. Sci. (Washington) 47 (1961), p. 1689.
7. J. R. Robinson, Biochem. J., 45 (1949), p. 68.
8. K. Scherrer and J. E. Darnell, Biochem. biophys. Res. Commun., 7 (1962), p. 486.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.