

THE EFFECT OF ANTIRONIDASE* SERUM OF GOATS ON THE METASTASIZATION OF BROWN-PEARCE CARCINOMA IN RABBITS

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According to the investigations of a number of authors [1, 3, 5, 7, 9, 13], malignant tumors and their adjacent tissues [3, 7] contain more than the normal quantity of spreading factor of the type of the enzyme hyaluronidase. The most intensive hyaluronidase activity is shown by metastases of malignant neoplasms [5, 6]. The addition of hyaluronidase to inoculated tumor material [2, 10], the injection of this enzyme into the tumor or near it [4, 14], and also the action of the enzyme on the body as a whole [8, 12] promotes the more intensive growth of the primary tumor and stimulates metastasis formation.

The necessity thus arises for the search for methods of inactivation of the spreading factor of malignant tumors. One such method may be the use of "antifactor" sera. Very little work, however, has been done on the study of this problem. For instance, it has been shown by experiments in vitro [2] that the activity of the hyaluronidase of Ehrlich's adenocarcinoma is depressed under the influence of an antitumor serum.

It has been reported [11] that the incidence of recurrence in mice, after extirpation of spontaneous tumors of the mammary glands, is decreased by administration of antihyaluronidase serum of rabbits.

The object of the present research was to study the action of the antihyaluronidase sera of goats on the process of metastasization of a Brown-Pearce carcinoma in rabbits.

EXPERIMENTAL METHOD AND RESULTS

The antihyaluronidase sera which we used in the experiments were obtained by the intravenous immunization of goats with a saline extract of rabbit's testis and of rodinase, and also a serum against the Brown-Pearce tumor, possessing antihyaluronidase activity. The sera were obtained on the 9-14th day after the last immunizing injection of extract.

The sera used in the experiments possessed the following titer: 1) the antironidase serum reacted with the

enzyme from the rabbit's testis in a dilution of 1:128 and with rodinase in a titer of 1:512; 2) the serum of the goat, immunized with extract of rabbit's testicular tissue, depressed the action of the homologous enzyme in a dilution of 1:512 and rodinase in a dilution of 1:64; 3) the titer of antibodies against the hyaluronidase of rabbit's testes in the serum of the goat, immunized with extract from tissue of a Brown-Pearce tumor, was 1:256, and against rodinase the titer was 1:16.

The experiments were conducted on 20 male chinchilla rabbits. Simultaneous intratesticular injections of 1 ml of a 25% suspension of cells from a Brown-Pearce tumor and from metastases in the liver and omentum were given to all the rabbits.

On the eighth day after inoculation of the tumor, the testes with the developing tumors were removed. After operation, the rabbits were divided in accordance with their weight into five equal groups with four rabbits in each.

On the day after removal of the tumor, i. e., on the ninth day after inoculation of the carcinoma, the rabbits were injected with sera. The rabbits of the first group received the serum of a goat immunized with rodinase. The animals of the second group received injections of the serum of a goat against extract of rabbit's testicular tissue. The third group of rabbits were injected with the serum of a goat immunized with Brown-Pearce tumor tissue. The fourth group were injected with normal goat serum. The animals of the fifth group received no serum.

The first injection of 6 ml of serum was made into the marginal vein of the ear. The next three injections of serum, in a dose of 5 ml each, were given into the thigh muscle. The interval between the first and sec-

* Ronidase is a preparation with hyaluronidase action, from the testis of the ox, marketed by the Mikoyan Meat Combine, Moscow.

TABLE 1. Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Primary Focus of the Tumor and Injection of Sera (21st Day of Transplantation of Tumor)

Group	Serum	Rabbit no.	No. of organs affected	Number of metastases ¹ present in one affected organ	Omentum		Spermatoc cord	
					degree of involvement	wt. (in g)	deg. of involvement	wt. (in g)
First	Against ronidase	2 973	—	—	—	5.0	—	1.5
		3 674	3	1	+	10.0	+	2.0
		2 190	5	7	+	8.5	+++	15.0
		2 402	4	15	+	12.0	+	5.0
		Mean	3.0	5.8		8.9		5.9
Second	Against rabbit's testes	14	2	16	+	7.5	—	1.5
		67	2	8	—	8.5	—	2.0
		3 741	11	270	+++	39.0	+++	15.0
		936		Died from accidental causes				
		Mean	5.0	98.0		18.3		6.2
Third	Against Brown-Pearce tumor	1 311	1	3	—	15	—	1.5
		1 028	10	32	+++	100	+++	26
		12	7	72	+++	35.5	+++	17
		2 939	9	141	++	20	+++	18
		Mean	6.8	62.2		42.6		15.6
Fourth	Normal goat	23	7	19	+	21	+++	18
		185	8	101	+++	41	++	11
		54	9	126	++	20	++	8
		3 065	11	653	+	7.5	+	6
		Mean	8.8	224.8		22.4		10.8
Fifth	No serum injected	1 369	8	13	++	33	++	9
		18	9	49	+	16	+	5
		46	6	127	++	26	+	8
		20	10	547	+++	82	+++	22
		Mean	8.3	184		39.3		11

Conventional signs: — metastases absent; + solitary metastases (from 1 to 10); ++ multiple metastases (from 11 to 50); +++ innumerable metastases (over 50).

¹Metastases in the spermatoc cord and omentum were disregarded when the number of metastases present in an affected organ was being counted.

and injections was 2 days, and between the others, 3 days. In order to avoid anaphylactic shock, this volume of serum in all cases was injected as fractionated doses. All the animals were sacrificed on the 21st day after inoculation of the tumor.

The effectiveness of action of the sera was assessed by the extent to which the organs were affected by metastases, taking into account the number of affected organs and the number of metastases in each.

The metastases were counted as follows. In organs containing a smaller number of metastases, those

on the surface were counted first, and later those in serial sections of the organs approximately 5 mm thick. The method of counting in organs containing a large number of metastases was rather more complicated. In these cases the number of metastases per unit weight of the organ was estimated, and then the total number for the whole organ was calculated. In organs in which it was not always possible to count the number of metastases, because the organ consisted of a conglomeration of confluent nodes (testis, omentum, etc.) the degree of involvement was designated conventionally

TABLE 2. Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Primary Focus of the Tumor and Injection of Sera (37th Day of Transplantation of Tumor)

Group	Serum	Rabbit no.	Number of organs affected	Number of metastases ¹ present in one affected organ	Omentum		Spermatic cord	
					degree of in- volve- ment	wt. (in g)	degree of in- volve- ment	wt. (in g)
First	Against ronidase	4	—	—	—	7	—	1.5
		55	—	—	—	18	—	1.5
		64	3	2	—	7	—	1.2
		42	5	3	+	7	++	12
		33	6	105	+	12	+	1.6
		38	8	136	+	14	++	14
Mean . . .			3.7	41.0		10.8		5.8
Second	Against Brown- Pearce tumor	37	7	2	+	7	+	7
		24	9	61	+	5	+	1.6
		36	11	141	+++	27	++	6
		62	8	176	+++	107	—	1.5
		78	8	275	++	8	++	9
		3	11	200	+++	117	+++	53
Mean . . .			9.0	142.5		45.1		13.0
Third	Normal goat	40	10	63	++	24	+++	20
		70	8	86	+++	53	+++	49
		35	11	446	+++	64	+++	53
		26a	11	629	++	8	+++	35
Mean . . .			10	306		37.2		39.2

Note: Legend as in Table 1.

by plus signs, and the degree of involvement was further compared in these organs by the gravimetric data.

The experimental results are shown in Table 1. The average number of organs affected by metastases, and the average number of metastases present in one affected rabbit's organ were least in the group of animals receiving antironidase goat serum.

Of each 12 organs examined, on the average three were affected, and for each affected organ in the rabbits of this group there were 5.8 metastases.

The injection of antitesticular and antitumor sera was almost equally effective, slightly lowering the incidence of affected organs (5 affected organs in the second group and 6.8 in the third) and the number of metastases in the organ (98 metastases in the second group and 62 in the third) by comparison with the control animals receiving nonimmune goat serum or no serum whatsoever (the respective values were 8.8 and 8.3 affected organs and 224.8 and 184 metastases in an affected organ).

When the gravimetric data were compared, the smallest values were also observed in the rabbits of the

first group, which received injections of antironidase serum [weight of omentum 8.9 g, mean weight of spermatic cord 5.9 g in the first group, compared with a mean weight of 22.4 g for the control groups (4 and 5), that of the omentum being 39.3 g and of the spermatic cord 10.8-11.0 g].

By comparison with the antironidase serum, the serum of the goat immunized against rabbit's testicles had almost the same effect on the development of the tumor in the spermatic cord of the second group of rabbits (weight 6.2 g).

The remaining sera had no significant effect either on the metastasization of the carcinoma in the omentum or on the development of the tumor in the spermatic cord.

The results obtained show that as a result of the action of antihyaluronidase immune sera on metastasization of a Brown-Pearce tumor in rabbits, the greatest depression of metastasization of the carcinoma was produced by the serum of the goat immunized with ronidase. In order to verify these results, in a second series of experiments 16 rabbits were used, divided into 3 groups

(6 rabbits in the first and second groups and 4 in the third group). The animals received injections as follows: first group — the serum of a goat immunized with ronidase; second group — a serum against Brown-Pearce tumor; third group — the serum of a normal, unimmunized goat.

For inoculation we used a 40% tumor suspension (0.5 ml). The testis with the tumor was removed on the seventh day after inoculation, and injection of the serum began on the eighth day, the same method being used as in the first experiment. The rabbits were kept under observation until the 37th day of development of the tumor, when those animals still alive were sacrificed. The effect of each serum was judged by the same criteria as in the first series of experiments (number of organs affected by metastases, number of metastases in an organ, and gravimetric data).

In this series of experiments too, the results obtained confirmed those of the preceding series. The least values of the degree of metastasization were observed in the first group of rabbits, receiving antironidase serum. In the experimental rabbits an average of from 3 to 7 organs was affected, compared with 10 in the controls (third group); an affected organ in the first group contained 41 metastases, compared with 306 in the third group. The same pattern was found for the organs for which the gravimetric data served as criteria of degree of involvement (weight of omentum in experimental series 10.8 g, control series 37.2 g; weight of spermatic cord in experimental series 5.8 g, control series 39.2 g). Some depression of the degree of metastasization was observed after injection of the antitumor serum (second group), as shown by a decrease in the number of metastases present in one affected organ and by a decrease in the weight of the spermatic cord by comparison with that in the control group (third group).

It must also be pointed out that among the animals of the first group no deaths had taken place on the 37th day, whereas in the control group, receiving normal goat serum, all the animals had died at this time (mean duration of survival of the rabbits of the control group was 28 days). Two rabbits of the group receiving antitumor serum were alive on the 37th day.

Thus the results of the second series of experiments also demonstrate a decrease in the degree of malig-

nancy of the tumor in the rabbits as a result of the injection of the serum of goats immunized with ronidase. We consider that the inhibiting action of antironidase sera on the metastasization of the carcinoma is due to the depression of hyaluronidase by the antibodies.

SUMMARY

It may be concluded from these experiments that the administration of an antironidase serum to rabbits after the preliminary removal of a primary Brown-Pearce tumor has an inhibiting effect on metastasization of the carcinoma. In our opinion this confirms the role of hyaluronidase in the process of metastasization of the tumor.

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