A COMPARATIVE INVESTIGATION OF THE ACTION OF PREPARATIONS FROM THE GROUP OF CHLORETHYLAMINES AND ETHYLENEIMINES ON EXPERIMENTAL HOMO- AND HETEROTRANSPLANTED TUMORS

A.M. Garin

Laboratory for the Experimental Chemotherapy of Tumors (Head – Corresponding Member of the AMN SSSR Prof. L. F. Larionov) and the Laboratory of Tumor Strains (Head-Doctor of Biological Sciences E.E. Pogosyants)* of the Institute of Experimental and Clinical Oncology (Dir.–Active Member of the AMN SSSR, Moscow
(Presented by Active Member of the AMN SSSR A. D. Timofeevski)
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Contemporary oncology deals with only a small number of the experimental forms equivalent to the neoplasias of the human. This essentially complicates the search for new active antitumoral preparations. Therefore, the interest and the attempts of a number of investigators to use the method of heterotransplantation of human tumors for experimental chemotherapy is understandable.

Thanks to the use of influences suppressing the immune powers of the recipient organism (cortisone, roentgen irradiation), it has been possible, in the last few years, to transplant human tumors – sarcomas and carcinomas – to hamsters and rats for prolonged periods, and to pass them serially over periods for several months and years [2,10,14,15].

In the years 1956-1958 there appeared the first short reports on the action of various chemopreparations on animals with tumores of human derivation [9,11,12,13].

It was established in the experiments of these authors that the different chemotherapeutic agents act variously on the heterotransplants of the same human tumor.

In the construction of therapeutic experiments on animals with heterotransplanted tumors it is possible to anticipate three variations in the results:

1. Tumors existing under unfavorable conditions within the alien organism (deteriorating blood supply, immunological effects) become vulnerable to any influences. In this case tumor heterotransplants are a poor means for the selection of new active antitumoral preparations or for the study of their spectra of activity.

2. Tumors developing resistance to the preparations to which they were sensitive in the original host. Resistance of this type could be explained by a change in the biological properties of the heterogenic tumor. In this case, investigating various preparations, we would not be able to elucidate their real activity.

3. The sensitivity of the tumors to the chemopreparations being preserved or changing minimally subsequent to heterotransplantation. A result of this type would speak in favor of the approach involving laborious investigations on heterotransplantations of tumors from the point of view of using them for experimental and clinical chemotherapy.

The purpose of this work was a comparative study of the action of certain preparations on several animal tumors under conditions of homo- and heterotransplantation. In the experiments we used a number of compounds from the group of chlorethylamines and ethylenimines, whose antitumoral activity, as well as methods of administration and dosages in association with homotransplantation, were thoroughly studied by coworkers in the Laboratory of Experimental Chemotherapy of the Institute of Experimental and Clinical Oncology, AMN SSSR [3,5,6,7].

EXPERIMENTAL METHODS

Many authors have studied the heterotransplantation of experimental tumors. They have obtained contradictory results. The results of the majority of these investigators are presented in the reviews of the literature by Yu. M. Vasil'ev [1] and Ahlstrom [8]. We considered it necessary to ourselves to study the following tumors, the potential and rapidity of their growth under the conditions of the alien organism, their weight and dimension: 1) rat tumors, transplanted to mice receiving cortisone: sarcoma M-1, sarcoma 45 (typical form and form resistant to sarcolysin), sarcoma SSK, Gehren's carcinoma, Walker's carcinosarcoma, ascitic hepatoma (strain AGF); 2) mouse tumors, transplanted to rats receiving cotisone: Ehrlich's ascitic tumor , lymphosarcoma – strain LIO-1 , two strains of mammary gland cancer – RSM and RMZh, Crocker's sarcoma, squamous cell cancer of the esophagus – strains OZh-1 and OZh-5, sarcoma 298, hepatoma 22; 3) Brown-Pearce's tumor of rabbits, transplanted to rats and mice receiving cortisone.

A concise characterization of the strains of experimental tumors used by us is contained in the review by E. E. Pogosyants [4].

* Yu. M. Vasil'ev, our elder scientific coworker, took part in the direction of the work.

All of the mouse tumors were transplanted, by means of cell suspensions, to nonpedigreed rats, 3-4 weeks of age: the rats tumors were transplanted to nonpedigreed mice, 15-20 grams in weight; the Brown-Pearce rabbit tumor was transplanted to mice of the S_3Na line, 15-20 grams in weight, and to nonpedigreed rats, 3-4 weeks of age (30-40 grams in weight). The site of transplantation for all the tumors was the subcutaneous cell layer of the left flank.

The following tumors, subsequent to heterotransplantation, usually did not exceed the measurements of a pea and became necrotic early: sarcoma M-1, sarcoma 45 (typical form and form resistant to sarcolysin), sarcoma SSK, Gehren's carcinoma, Walker's carcinosarcoma, ascitic hepatoma AGF, lymphosarcoma LIO-1, Ehrlich's ascitic tumor, squamous cell cancer of the esophagus OZh-1 and OZh-5, hepatoma 22.

Adequate growth, subsequent to heterotransplantation to rats, was yielded by the following mouse tumors: mammary gland carcinoma (strains RSM and RMZh), Crocker's sarcoma and sarcoma 298, and also Brown-Pearce's rabbit tumor (with transplantation to mice and rats). We used these strains in our therapeutic experiments. The effect of the following preparations was studied: embichin, i.e. methyl-di-(2-chlorethyl)-amine, in a single dose of 0.15 mg/kg for rats and mice; sarcolysin, i.e. DL-p-di-(2-chlorethyl)-amino-phenylalanine, in doses of 0.5 mg/kg, 1.5 mg/kg, and 2 mg/kg; metasarcolysin fluoride, i.e. 2-fluoro-5-di-(2-chlorethyl)-aminophenylalanine, in a single dose of 1.6 mg/kg for rats, 5 mg/kg for mice, and 4 mg/kg for rabbits; benzodate, i.e. 2-benzyloxy-4,6-diethyleneimino-1-3-5-triazine, in doses of 8 mg/kg for rats, 15 mg/kg for mice; thio-TEPA (triethylenethiophosphoramide), in doses of 2.5 mg/kg for rats and 4 mg/kg for mice; phenamete, i.e., di-(2-chlorethyl)-aminophenacetyl-methionine ethyl ether, in doses of 20 mg/kg for rats and 110 mg/kg for mice. Because of the varying tolerance of the rats, mice and rabbits to the preparations employed by us, we used them in the maximum tolerable doses.

Tumor therapy using these preparations began on the 5th day following heterotransplantation and continued over the course of 7-8 days, after which the animals were sacrificed, using ether. All the preparations were administered intraperitoneally, with the exception of phenamete, which was introduced into the stomach. The animals received benzodate at intervals of 72 hours; the remaining preparations were administered daily. The average diameter of the tumors in the control and experimental groups were measured on the 5th, 8th and 12th day after transplantation. At the end of the experiment the percent inhibition of tumor growth was calculated by the average weight of the tumors of each group. All the animals were injected with cortisone twice a week – 2.5 mg for the rats, and 2 mg for the mice. For comparison and as control, therapeutic experiments were set up simultaneously on homotransplants of these same tumors. Treatment with the preparations in these experiments continued for 10-12 days.

The data obtained are presented in the table. As can be seen, sarcoma 298, subsequent to homotransplantation, was sensitive to phenamete and sarcolysin (89 and 82 percent inhibition). The high degree of sensitivity of this tumor was preserved in association with heterotransplantation as well (95-89% inhibition). Sarcoma 298, under the conditions of homotransplantation, was absolutely insensitive to embichin. The same observation was made with heterotransplantation of the tumor to rats.

Crocker's sarcoma in mice was sensitive to benzodate in a dose of 15 mg/kg (95% inhibition); under conditions of heterotransplantation to rats that preparation gave rise to 68% inhibition of tumor growth in a dose of 8 mg/kg. A certain reduction in the antitumoral effect possibly depends on the smaller dose and the shortened interval of treatment. Using sarcolysin in doses of 2 and 1.5 mg/kg, the growth of Crocker's sarcoma in mice was inhibited by 59 and 50%. With heterotransplantation of the tumor to rats, sarcolysin in these doses gave rise to 66 and 64% inhibitions respectively. Sarcolysin, in a dose of 0.5 mg/kg, did not effect the growth of Crocker's sarcoma, either in mice or in rats.

Strain RMZh (cancer of the mammary gland) was essentially not inhibited by the application of sarcolysin and phenamete either in homo- or heterologous transplantation.

Sarcolysin fluoride showed itself to be a preparation strongly influencing the growth of another mammary gland carcinoma (RSM) under conditions of homo- and heterotransplantation (97 and 81%). Embichin and thio-TEPA did not act on this tumor in mice; in rats, thio-TEPA caused a stimulation in the growth of Crocker's sarcoma, while embichin did not evidence a notable effect.

Preservation of the sensitivity of the Brown-Pearce tumor to sarcolysin and metasarcolysin fluoride was observed in the case of transplantation of that strain to animals belonging to two different genuses. Thus, metasarcolysin fluoride yielded 94% inhibition of the growth of that tumor with subcutaneous transplantation of it to rabbits, 81% inhibition with the tumors growing in mice, and 82% in rats. Sarcolysin (in doses of 3 mg/kg after 72 hours in the experiments on rabbits and 1.5 mg/kg daily in mice and rats) did not give rise to a statistically significant inhibition in the growth of this tumor, neither with homologous nor heterologous transplantation.

In conjunction with histological investigations of the heterotransplanted tumors, a morphological picture was observed identical with that of the original. In tumors treated with the active preparations there were noted degenerative changes, up to necrosis of the tumor cells, increase in the amount of connective tissue stroma, and lymphoid and leukocytic infiltration. The detailed description of the microscopic picture of the treated heterogenic tumors will be the subject of a special report.

Thus, the investigation carried out showed that the degree and character of sensitivity of tumors of mice (mammary gland carcinomas, strains RSM and RMZh, Crocker's sarcoma, sarcoma 298) and Brown-Pearce's

Results of the Therapeutic Experiments

Tumor	Preparation	Homotransplantation: number of animals	dose of the preparation (in mg/kg)	average weight of the tumor (in mg)	percent inhibition	criteria R*	Heterotransplantation: number of animals	dose of the preparation (in mg/kg)	average weight of the tumor (in mg)	percent inhibition (-) or stimulation (+)	criteria R*
Mouse sarcoma 298	Control	10	—	1 566	—	—	10	—	1 995	—	—
	Phenamete	10	110	166	89	1	10	20	115	—90	1
	Control	10	—	1 566	—	—	14	—	1 943	—	—
	Sarcolysin	10	1,5	270	82	1	15	1,5	202	—89	1
	Phenamete	10	110,0	166	89	1	17	20,0	94	—95	1
	Control	10	—	1 566	—	—	15	—	935	—	—
	Sarcolysin	10	1,5	270	82	1,0	15	1,5	66	—93	1,0
	Embichin	9	0,15	1 477	5	0,44	14	0,15	1 050	+12	0,621
Crocker's mouse sarcoma	Control	5	—	5 190	—	—	14	—	7 339	—	—
	Benzodate	6	15	343	95	0,998	18	8,0	2 332	—68	1
	Sarcolysin	—	—	—	—	—	13	1,5	3 336	—54	1
	Control	10	—	3 850	—	—	15	—	2 056	—	—
	Sarcolysin	9	2,0	1 544	59	0,999	13	2	688	—66	0,998
	"	9	1,5	1 888	50	0,991	—	—	—	—	—
	"	9	0,5	3 311	19	0,6	14	0,5	2 623	+27	0,715
Mouse carcinomas RMZh	Control	10	—	955	—	—	10	—	1 400	—	—
	Sarcolysin	9	1,5	755	19	0,669	9	1,5	1 372	—2	0,236
	Phenamete	8	110,0	537	43	0,872	10	20,0	1 032	—26	0,669
Mouse carcinomas RSM	Control	10	—	2 060†	—	—	19	—	2 714	—	—
	Thio-TEPA	10	4	1 680	18	0,619	18	2,5	3 838	—41	0,978
	SF**	—	—	—	—	—	18	1,6	491	—81	1
	Control	10	—	2 255	—	—	19	—	3 271	—	—
	SF**	10	5,0	52	97	1,0	—	—	—	—	—
	Embichin	9	0,15	2 211	2	0,3	16	0,15	2 798	—14	0,6
Brown-Pearce tumor in rats	Control	5‡	—	2 850	—	—	10	—	3 210	—	—
	SF**	5	4	140	94	0,998	10	1,8	570	—82	0,999
	Sarcolysin	5	3 over 72 hr	2 440	5	0,4	10	1,5	2 280	—29	0,669
Brown-Pearce tumor in mice	Control	—	—	—	—	—	10	—	411	—	—
	BPT††	—	—	—	—	—	9	5	75	—81	1,0
	Sarcolysin	—	—	—	—	—	9	1,5	350	—14	0,617

* Criteria R of less than 0.975 indicates a non-significant difference from the control. † The experiment was set up jointly with S. A. Papoyan.
‡ The tumor was transplanted under the skin. ** SF=Sarcolysin fluoride. †† BPT=Brown-Pearce tumor.

rabbit tumor to preparations of the chlorethylamine and ethylenimine groups (embichin, sarcolysin, metasarcolysin fluoride, phenamete, benzodate, thio-TEPA) is preserved with heterotransplantation to rats.

SUMMARY

The author studied the effect of some antiblastic chemotherapeutic preparations on the growth of mouse tumors (Crocker's sarcoma, cancers RSM and RMZh of the mammary gland, and sarcoma 298) and rabbit Brown-Pearce tumor in heterotransplantation. Mouse tumors were inoculated into three- and four-week-old rats, and rabbit tumors were inoculated into rats and mice. Cortisone was administered to the recipient animals. Therapeutic experiments were conducted on homotransplants of the same tumors. It was shown that the homo- and the heterotransplanted tumors were approximately identical in sensitivity to chemotherapeutic preparations investigated (embichin, sarcolysin, thio-TEPA, etc.).

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