INCREASED RATE OF GROWTH OF TRANSPLANTED TUMORS AND CHANGES IN THEIR SENSITIVITY TO CHEMOTHERAPY INDUCED BY CREATININE

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Daily subcutaneous injections of creatinine in a dose of 10-20 mg/kg accelerated growth of certain transplanted tumors in rats and mice. Creatinine also modified the effect of antitumor preparations irrespective of their structure.

Creatinine (1-methyl-2-imidohydantoin), which occurs in man and animals as an end metabolite, has the property of forming complexes, which is made use of in the preparation of the complex salt of serotonin: 5-hydroxytryptamine creatinine-sulfate [3, 8]. The creatine salt of serotonin has a much weaker action on Ehrlich's carcinoma and on sarcoma 37 than the hydrochloride when used in a dose equimolar as regards its 5-hydroxytryptamine content [2].

This last observation suggested that creatine might also have some effect on factors determining the sensitivity of tumors to chemotherapy. Experiments to test this hypothesis are described below.

EXPERIMENTAL METHOD

Creatinine (Chemapol, Czechoslovakia) was injected subcutaneously in physiological saline daily for 12-14 days into rats and mice with transplanted tumors, starting on the 5th-7th day after inoculation of the tumor. Every four days during the experiment the tumor was measured and its mean diameter calculated. At the end of the experiment the tumors were weighed and the difference between their mean weight in the experimental series and untreated control was determined and expressed as a percentage of the mean weight of the tumors in the control. In another group of experiments, creatinine was injected before injection of 5-hydroxytryptamine (serotonin) or sarcolysin. The serotonin was given as two different salts: the hydrochloride and the creatinine-sulfate. Both compounds were obtained by M. F. Petrova in the writers' institute. Each group (experimental and control) contained ten animals. The material was analyzed by the Student–Fisher method.

EXPERIMENTAL RESULTS

Preliminary experiments showed that creatinine has no marked toxic or local irritant action on rats when given as a single injection in the doses specified above. However, after repeated intraperitoneal, and sometimes subcutaneous, injection of 20 mg/kg, especially in August rats, some decrease in weight, apathy, diarrhea, and occasional (10-13%) death of the animals were observed after a course of injections. Postmortem examination usually revealed edema of the parenchymatous organs, the intestine filled with fluid contents, and some degree of ascites. Administration of the compound into the stomach caused no changes in the internal organs and was followed by an increase, not a decrease, in the rats' weight. The results of experiments on rats and mice with different tumors showed that repeated subcutaneous injection of creatinine causes a marked increase in the mean diameter of the tumors, accompanied by a corresponding increase in their mean weight over the control (Table 1), although the difference between the mean

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TABLE 1. Effect of Creatinine on Tumor Weight and on Life Span of Animals with Leukemia

Expt. No.	Type of tumor	Line and sex of animals*	Daily dose (in mg/kg)	Mode of administration	Mean weight of tumors (in g)		Difference in weight of tumors (as % of control)
					control	experiment	
			Rat tumors				
1	Jensen's sarcoma	n-i ♂	10	Subcutaneously	43.1	58.2	26
2	" "	n-i ♀	15	"	32.0	39.0	22
3	Walker's carcinosarcoma: slow-growing	n-i ♂	13	"	9.7	24.2	150
4	fast-growing	n-i ♀	15	"	43.2	44.8	4
5	Sarcoma 45	n-i ♀	20	"	30.2	33.0	10
6	Ditto	n-i ♀	20	By gastric tube	30.2	22.7	−10
			Mouse tumors				
7	Sarcoma 180	n-i ♂	15	Subcutaneously	6.5	7.8	20
8	Harding–Passey melanoma	C57Bl ♂	20	"	3.8	4.8	27
9	Sarcoma 37	n-i ♀	10	"	5.8	5.1	0
10	Ditto	n-i ♂	20	"	10.8	10.9	0
			Leukemias and ascites tumors of mice				
					Number of days surviving		Difference (in %)
11	Leukemia L1210	DBA/2 ♀	20	Subcutaneously	7.5	6.4	15
12	Leukemia La	C57Bl ♂	15	"	8.8	8.2	0.7
13	Ehrlich's tumor	C57Bl ♀	20	"	21	14	33

*N-I denotes noninbred.

weight of the tumors from the control was not statistically significant ($P > 0.05$) except in experiment No. 3. In leukemias L1210 and La, and also in Ehrlich's ascites carcinoma, the mice died on the average earlier in the experiments with creatinine than in the control. In the experiments (Table 2) to determine the role of creatinine in the action of its complex salt with serotonin on the tumor, in agreement with the results obtained by Scott [9, 10], it was found that the complex stimulates the growth of Walker's carcinosarcoma 256. Nevertheless, in the same dose (20 mg/kg), this salt inhibited growth of Jensen's sarcoma (experiments Nos. 3 and 7). Serotonin hydrochloride, when given in a dose equimolecular in its 5-hydroxytryptamine content, inhibited growth of both tumors, but it acted much more strongly on Jensen's sarcoma than serotonin creatinine-sulgate.

In experiment No. 6 (Table 2), rats with Jensen's sarcoma received daily subcutaneous injections of both amines separately, creatinine as the base, and serotonin as the hydrochloride 20 min later. As a result, potentiation by creatinine of the inhibitory action of serotonin was unexpectedly obtained: the mean weight of the tumor in this group was only one-quarter that in the simultaneous experimental group of animals receiving 5-hydroxytryptamine hydrochloride only and only one-ninth of that in the group receiving the creatinine salt of serotonin. In an attempt to determine whether the ability of creatinine to modify antitumor action is specific to serotonin, the serotonin was replaced in analogous experiments with creatinine by a compound of a completely different chemical group, namely sarcolysin (Table 3), yet the identical result was obtained. Creatinine increased the specific activity of sarcolysin by 63%, a significant difference. However, the results of the experiments with sarcoma 45 show that under the same conditions creatinine can "protect" sarcoma 45 against the action of sarcolysin, weakening the effect of the compound by 49–72% (as reflected in the weight of the tumor), and by 41–27% in the case of sarcoma 180.

TABLE 2. Effect of Creatinine on Antitumor Activity of 5-Hydroxytryptamine (HT) in Noninbred Rats

Expt. No.	Type of tumor	Compound	Dose (in mg/kg)	Mean weight of tumor			Difference in weight (in % of control)	
				control	experiment	P	inhibition of growth	stimulation
1	Walker's carcinosarcoma 256	HT hydrochloride	10	24.4	13.5	0.05	45	–
2	Ditto	" "	20	48.9	8.5	0.001	83	–
3	"	HT creatinine-sulfate	20	33.0	45.6	0.05	–	39
4	"	Creatinine	15	43.2	44.8	<0.05	–	4
5	Jensen's sarcoma	HT hydrochloride	10	43.1	5.8	0.001	87	–
6	" "	Creatinine + HT hydrochloride	10	43.1	1.5	0.001	97	–
7	" "	HT creatinine-sulfate	20	43.1	13.4	0.001	71	–
8	" "	Creatinine	10	43.1	53.2	0.05	–	26

TABLE 3. Protection of Sarcoma 45 and Sarcoma 180 against the Action of Sarcolysin and Sensitization of Walker's Carcinosarcoma to Its Action after Preliminary Administration of Creatinine

Expt. No.	Type of tumor	Compounds: sarcolysin (S), creatine (C), and their (in mg/kg)	Interval (in min) between administration of compounds	Mean weight of tumor (in g)	P relative to control with sarcolysin	Difference in weight of tumor (in % of control with sarcolysin) doses
1	Sarcoma 45	Control: S. 0.75	–	11.2	–	–
2	Ditto	C. 20 + S. 0.75	30	16.7	0.1	49
3	"	Control: S. 1.5	–	6.0	–	–
4	"	C. 20 + S. 1.5	30	10.3	0.02	72
5	"	Untreated control		30.2	–	–
6	Walker's carcinosarcoma	Control: S. 0.75	–	7.9	–	–
7	Ditto	C. 15 + S. 0.75	30	2.9	0.001	63
8	"	Untreated control	–	41.6	–	–
9	Sarcoma 180	Control: S. 2	–	2.2	–	–
10	Ditto	C. 20 + S. 2	30	3.1	0.05	41
11	"	C. 20 + S. 2	15	2.3	–	–
12	"	C. 20 + S. 2	5	2.8	0.05	27
13	"	Untreated control	–	6.5	–	–

The modifying effect of serotonin is thus evidently specific relative to the antitumor preparation and, consequently, it is connected with certain changes in the tumor, possibly to do with the cell cycle. The reason why different workers studying different transplanted tumors obtained conflicting results with the creatinine salt of serotonin is thus easy to understand. For instance, Scott [9, 10] observed an increase in the weight of growth of some transplanted tumors under the influence of the creatinine salt of serotonin and concluded that serotonin has an important pathogenetic role as stimulator of tumor growth. Other workers [1, 2, 5-7, 11] found that serotonin has an inhibitory action. Creatinine, which passes extremely readily through cell membranes [4], requires detailed study from the point of view of its action on the tumor cell.

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