INHIBITION OF GROWTH OF MOUSE TUMORS BY INJECTIONS OF SEROTONIN OR SEROTONIN AND HISTAMINE COMBINED

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IN a previous publication[1] it was noted that daily injections of serotonin creatinine sulfate into tumors of mice "cured" 7 of 55 $B_6D/2F_1$ hybrid mice with S91 melanomas implanted on their feet, and caused striking inhibition of the growth of the tumors in all treated mice. Pukhalskaya[2] also has observed antitumor effects of serotonin, noting that serotonin hydrochloride caused a significant inhibition in a number of animal tumors as well as an inhibition of cell division in in vivo experiments on the regenerating tadpole tail and rat cornea. Pukhalskaya stated that serotonin must be used in the form of the hydrochloride rather than the creatinine sulfate salt, and emphasized that it must be injected hypodermically and not intraperitoneally. The dosage employed was 20 mg. per kilogram of body weight per day for eight days.

Additional observations on the regression of mouse tumors after injections of serotonin are presented in the following report.

MATERIALS AND METHODS

Four types of tumors were minced and transplanted to the webs of the left hind feet of five types of mice as follows:

1. S91 melanoma in DBA_1 or $B_6D/2F_1$ hybrid mice, male or female, 6 weeks old.
2. Sarcoma 180 in female Swiss mice, 6 weeks old.
3. T241 sarcoma in male or female C57BL/6 mice or in $B_6D/2F_1$ hybrids, 6 weeks old.
4. Sarcoma 1 in strain A mice, 6 weeks old.

The natural history of these tumors has been discussed in detail elsewhere.[1] Sarcoma 1 grows rapidly when implanted on the hind feet of strain A mice, and metastasizes almost exclusively to lymph nodes. It is an isologous tumor that is not rejected by the strain A host. S91 is isologous in DBA_1 mice and once established is not rejected. In $B_6D/2F_1$ hybrid mice spontaneous regression occurs in about one in fifty mice. Sarcoma 180 is homologous in Swiss mice and often regresses spontaneously. T241 in C57BL/6 or $B_6D/2F_1$ hybrids is isologous and does not regress.

Serotonin was injected into the tumors in the form of serotonin creatinine

sulfate* diluted so that 2.5 mg. was contained in 0.1 ml. of warm distilled water. Each weekday 0.1 ml. of this solution was injected into each of the tumors. The treatment was started when the tumors were just beginning to assume a round shape (weight of tumor 5 to 10 mg.), and was continued for from 2 to 4 weeks. An alternative method of treatment was a once-weekly injection through a 20-gauge needle, of "serotonin paste," a thick, concentrated suspension containing 25 mg. of serotonin creatinine sulfate crystals per 0.1 ml. of water. The tumors of the control mice were injected with 0.1 ml. of balanced saline solution every time the experimental mice were treated with a serotonin preparation. A mouse was considered to be "cured" when two months after beginning of treatment there was no visible tumor. Often in mice that had had melanomas, there was a residual dark stain on the foot, but sections of these stains showed that the pigmentation was due to melanin in macrophages and that there was no residual tumor.

RESULTS

Daily injections of 2.5 mg. of serotonin solution in 0.1 ml. of water into the webs of normal mice's feet did not appear to damage the feet and were well tolerated by the mice.

Injection of serotonin into the tumors inhibited the growth of all types of tumors studied, but "cures" were effected only in mice with S91 melanomas. Of 65 treated mice bearing S91 melanomas, 11 remained free of tumor for periods averaging 3½ months (*Table 1*) before they were killed. Even when "cures" were not effected, the growth of the tumors was strikingly inhibited and survival of the mice was prolonged, 22 of 65 surviving as compared with 10 of 65 control mice.

Systemic treatment by intraperitoneal injection of 7.5 mg. of serotonin daily did not affect the growth of S91 melanoma, nor were tumor cells of S180 killed by exposure to serotonin in vitro. Two and one-half milligrams of serotonin creatinine sulfate in 0.2 ml. of water added to 0.2 ml. of a one-half dilution of cytosieved sarcoma 180 cells and incubated for ½ hour at room temperature did not interfere with the growth of these cells when they were transplanted into Swiss mice.

Injection of a thick suspension of undissolved serotonin crystals into the tumors twice a week inhibited the growth of the tumors and cured one of nine DBA_1 mice with S91 melanomas. None of the control animals was free of tumor. Hourly injections of serotonin solution (2.5 mg. per 0.1 ml. of water) for 6 hours on one day a week caused striking inhibition of the growth of S91 melanoma in $B_6D/2F_1$ hybrid mice. The tumor-bearing feet were amputated and their weights were compared 36 days after the injections were started. Four normal feet weighed 0.60 gm.; four serotonin-in-

* *Supplied through the courtesy of The Upjohn Company, Kalamazoo, Michigan.*

Table 1.–*Summary of eight experiments in which $B_6D/2F_1$ hybrid mice or DBA_1 mice bearing S91 melanoma on left hind feet were treated by daily intratumor injections of 2.5 mg. of serotonin creatinine sulfate. Controls were observed for the same periods of time and were treated by intratumor injections of saline solution.*

Experiment no.	Type of mouse	Sex	Number of mice	Number "cured"	Number of months observed	Number of control mice	Number of control mice "cured"
1	Hybrid	M	6	2	6	6	0
2	Hybrid	F	8	0	4	7	0
3	Hybrid	F	10	3	6	10	0
4	Hybrid	M	11	2	2	11	1
5	Hybrid	F	7	0	2	7	0
6	Hybrid	M	4	3	2	4	1
7	DBA_1	M	8	1	2	8	0
8	DBA_1	M	11	0	4	10	0
	Total		65	11	(av. 3.5 mo.)	63	2

jected, tumor-bearing feet weighed 0.96 gm., and four saline-injected tumor-bearing feet weighed 5.85 gm.

Pilot studies were made, utilizing 10 to 20 mice in each experiment. When hyaluronidase was added to a suspension of serotonin and was injected into S91 melanomas in the feet of DBA_1 mice, most of the mice died, apparently as a result of too rapid absorption of a toxic dose of serotonin. Suspension of serotonin in glycerol instead of water also was toxic. Serotonin suspended in sesame oil was ineffective. Addition of heparin did not increase the inhibitory effect of serotonin on the growth of tumors.

Daily injections of serotonin solution or biweekly injections of a suspension of serotonin into sarcoma 180 implanted in the feet of Swiss mice inhibited the growth of the tumors. It often destroyed the part of the tumor that was on the web of the foot, but failed to control extension of the tumor up into the leg. Serotonin was no more effective than saline in effecting permanent control of sarcoma 180. The same was true of treatment of T241 on the feet of C57BL/6 mice, and the incidence of pulmonary metastasis from these tumors was not affected by the treatment. Seven and one-half milligrams of serotonin injected daily intraperitoneally into Swiss mice with Ehrlich ascites tumors neither inhibited formation of ascites nor prolonged life.

Sarcoma 1 in strain A mice was well controlled locally by injection of serotonin into the tumor, but the tumor had a striking tendency to spread up the leg, or to metastasize to regional nodes, so that all the mice even-

tually died of recurrence or metastasis. Injection of a suspension of 7.5 mg. of serotonin in 0.2 ml. of sesame oil into involved inguinal or popliteal nodes averaging 1 cm. in diameter caused complete regression of the tumor in the injected nodes of 4 of 6 mice for the duration of their lives (average, 3 weeks). Likewise, daily injections of 5 mg. of serotonin solution into nodes from 1 cm. to 1.5 cm. in diameter involved with sarcoma 1 caused regression in four of four mice. All later died of other metastases.

Injection of alcohol, croton oil, and other destructive substances into the tumors on the feet or in the nodes did not cause selective destruction of the tumors without destruction of the feet. The pH of the serotonin solution was not a factor in the inhibition of the growth of the tumors, because hydrochloric acid solution of the same pH (3.2) as that of the serotonin solution did not inhibit the growth of the tumor.

DISCUSSION

Tumors implanted on the feet of mice tend to ulcerate, even when untreated, and when they do, it is no longer possible to inject liquid into them and have it retained. The incidence of complete regression and "cure" might have been higher if the tumors had not ulcerated and allowed the solutions to leak out. Despite leaking, serotonin effected a striking inhibition of the rate of growth of S91 melanoma and resulted in gross and histologic disappearance of the tumors of 11 of 65 mice.

Daily intratumor injections of 1.25 mg. of histamine mixed with 1.25 mg. of serotonin in 0.1 ml. of water caused striking inhibition of the growth of S91 melanoma and cured 18 of 25 DBA_1 mice (living and free of cancer for three months). Two and one-half milligrams of histamine injected daily into the tumor of each of 25 mice cured none and caused little, if any, inhibition of the rate of growth. Two and one-half milligrams of serotonin alone cured only 11 of 65 mice. Serotonin and histamine given together in doses of 1.25 mg. of each were thus more effective than 2.5 mg. doses of either histamine alone or serotonin alone.

Since serotonin and histamine are thought to be present in inflammatory reactions, it is possible that their release plays a part in the so-called spontaneous regressions of tumors that have been observed after inflammation.

SUMMARY

Serotonin in concentrated solution, or suspension, injected repeatedly into S91 melanomas implanted on the feet of DBA_1 or $B_6D/2F_1$ hybrid mice inhibited the growth of all tumors and selectively destroyed some of them without damaging the feet.

Intratumor injections of serotonin destroyed metastases of sarcoma 1 in the lymph nodes of strain A mice.

Repeated intratumor injections of serotonin inhibited the growth of sarcoma 180, T241, and sarcoma 1 on the feet of mice, but did not prevent metastasis or effect cures.

Repeated intratumor injections of a solution containing both serotonin and histamine were more effective than serotonin alone, and resulted in complete control of S91 melanoma implanted on the feet of 18 of 25 DBA_1 mice. Injections of histamine alone failed to inhibit the growth of the tumors.

References

1. Crile, G., Jr.: The effects of heat and radiation on cancers implanted on the feet of mice. Cancer Res. **23:** 372–380, 1963.

2. Pukhalskaya, E. C.: Mechanism of antimitotic and antitumor action of 5-hydroxytryptamine (serotonin). Acta Un. Int. Cancer **20:** 131–134, 1964.