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Effects of Hadacidin on Human Tumors Grown in Eggs and Rats.* (27402)

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Transplantable human tumors have been used in experimental chemotherapy studies for a number of years. The reports in the literature attest to the value of transplantable human tumors in primary screening and other drug evaluation studies, using chick embryos(1-7) and rodents(8-11) as hosts.

The studies presented here are concerned with evaluation of a new substance of microbial origin, characterized as N-formyl hydroxyaminoacetic acid and given the trivial name hadacidin(12). The anti-tumor properties of hadacidin were first demonstrated in tests which resulted in consistent inhibition of transplantable human tumors, intestinal adenocarcinoma (H. Ad. 1) and epidermoid carcinoma (H. Ep. 3), growing on the chorioallantoic membrane (CAM) of chick embryos(13).

The results presented here confirm the activity of hadacidin against H. Ad. 1 and H. Ep. 3 growing in embryonated eggs, and extend those findings to include the activity of hadacidin against 2 additional transplantable human tumors, a bronchogenic carcinoma (A-42) and a sarcoma (H.S. 1), growing on the CAM of chick embryos. In addition, this

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TABLE I. Effect of Hadacidin (Sample 1) on Human Tumors in Eggs.

Tumor	Dose, mg/egg	Mortality T	Mortality C	Avg tumor wt (mg) T	Avg tumor wt (mg) C	Results, % inhibition
H. Ad. 1	5.00	0/6	0/7	363	1021	64
	5.00	1/6	0/8	321	480	33
A-42	5.00	2/6	1/8	1170	3401	66
	5.00	1/6	1/8	492	1937	75
H. Ep. 3	5.00	1/6	1/8	640	1038	38
	5.00	1/6	0/6	450	855	47
H. S. 1	1.50	1/6	0/8	803	755	0
	1.00	1/6	2/8	1003	944	0

report deals with the testing of hadacidin against H.S. 1, growing in the x-irradiated and cortisone treated rat. These results from the mammalian host serve to establish more critically the anti-tumor properties of hadacidin.

Methods. The procedures followed in the chick embryo studies are essentially the same as those previously described(5). The salient features of the procedures are presented here.

Fertile White Leghorn eggs, incubated for 9 days, were used in these studies. Each egg received a single tumor fragment implanted on the CAM. Four days after implantation the eggs were inspected for tumor growth to insure using only tumor bearing eggs in the experiments. Single-dose injections of hadacidin were made into the yolk sac of the treated embryos. Evaluations were made 6 days after injecting hadacidin. The 4 types of tumors used in these studies are Toolan's H. Ep. 3, H.S. 1, and H. Ad. 1(14,15), and Skiff's A-42(16).

The anti-tumor activity of hadacidin was determined by comparing mean tumor weights between treated and control groups to determine the extent of tumor inhibition in the experimental groups.

The experimental chemotherapy procedure using transplantable human tumors in the rat host has been described(9). The procedure is summarized here.

Weanling female Wistar or Sprague-Dawley albino rats were x-irradiated with a single total body dose of 150 r 1-3 days prior to subcutaneous inoculation in the flank with 0.5 ml of a 50% minced tumor suspension. The rats were given subcutaneous injections in the nape of the neck with doses of cortisone acetate equal to 60 mg/kg of body weight. The first dose of cortisone was given immediately after tumor inoculation, the remaining doses were given on alternate days until a total of 4 were administered. Therapy was begun, intraperitoneal or *per os*, with hadacidin 24 hours after tumor inoculation and continued for a total of 9 days. Hadacidin was prepared fresh daily in saline and administered either as a single dose daily (Sundays omitted) or in divided doses (dose/3) given 3 times a day. The rats were sacrificed on the 11th or 12th day after tumor inoculation. The tumors were excised, cleared of extraneous material and weighed. Comparisons were made between the mean weights of treated and control groups to determine the per cent of tumor inhibition.

Results. The first sample of hadacidin on which comparative studies in the egg were made was a mono sodium salt. These results are shown in Table I. In these tests embryos bearing H.S. 1 tumors would not tolerate 5.0 mg/egg of hadacidin as did embryos bearing other types of tumors, thus 1.50 mg/egg was the highest dose used against this tumor. Hadacidin was inactive against H.S. 1 at 1.50 mg/egg, while being active against the other 3 types of tumors at 5.0 mg/egg.

A second sample of hadacidin was again a mono sodium salt which was tested to determine the relative anti-tumor potency as compared with the previous sample. The results (Table II) indicate that the anti-tumor potency of this sample is comparable to that of the previous sample. At a dose level of 5 mg/egg, hadacidin was active against all of

TABLE II. Effect of Hadacidin (Sample 2) on Human Tumors in Eggs.

Tumor	Dose, mg/egg	Mortality T	Mortality C	Avg tumor wt (mg) T	Avg tumor wt (mg) C	Results, % inhibition
H. Ad. 1	5.00	1/6	0/8	407	838	52
	5.00	0/6	0/8	613	1066	42
	10.00	2/6	0/8	320	838	62
A-42	5.00	0/6	0/7	592	2111	72
	5.00	1/6	1/8	410	1425	71
	10.00	2/6	1/7	554	2309	76
H. Ep. 3	5.00	1/6	0/6	478	855	44
	5.00	1/6	1/8	423	845	50
H. S. 1	5.00	0/6	0/6	353	846	58
	2.50	0/6	0/6	450	792	43

the tumor types used in these tests in the embryonated egg.

Results of tests with the sodium salt of hadacidin against H.S. 1 in the x-irradiated and cortisone treated rat are shown in Table III. Three tests at 1500 mg/kg given intraperitoneally in single daily doses resulted in an averaged tumor inhibition of 61%. In a single test at 3000 mg/kg given intraperitoneally in single daily doses, tumor inhibition was 37%. Likewise a single test at 1500 mg/kg administered *per os* once daily resulted in 37% inhibition. Three tests at a dose level of 4000 mg/kg given *per os* in divided doses 3 times daily gave an averaged tumor inhibition of 67%. Similarly, a single test at the same dose level and using the same dosing schedule, but administered intraperitoneally instead of *per os,* gave 68% inhibition.

Discussion. In testing hadacidin in embryonated eggs, the confirmatory test against H. Ad. 1 with the first sample evaluated (Table I) showed only about one-half the tumor inhibition as did the initial test. Inspection of the Table shows the control tumor average of the confirmatory test to be less than one-half that of the control tumor average in the initial test. Past experience in drug evaluation has shown that differences among tests in control tumor sizes may result in similar differences among tests in per cent of tumor inhibition. The negative results obtained when the first sample of the sodium salt of hadacidin was tested against H.S. 1 are attributed to the failure of embryos bearing this tumor to tolerate higher doses of the compound. It is not uncommon for H.S.1 tumors to affect the embryos in some manner which prevents them from tolerating the same dose of a compound as do embryos bearing other types of tumors. This interference with drug tolerence produced by H.S. 1 appeared periodically and was not consistent with each transplant generation. This interference phenomenon has not been explained.

When the results of testing sample 1 are compared with those of sample 2 (Tables I and II) it becomes quite obvious that the relative anti-tumor potencies of the 2 samples are equal to each other. When the second sample of hadacidin was tested, the interference with drug tolerence of H.S. 1 tumors had diminished so that the embryos would tolerate 5 mg/egg. The ability of the embryos to tolerate a higher dose of hadacidin in these tests resulted in the demonstration of the inhibitory effect of hadacidin against H.S. 1 tumors. An inspection of Tables I and II clearly indicated that the bronchogenic carcinoma is consistently more sensitive to a given quantity of hadacidin than are the other types of tumors used in these tests.

Evaluation of the sodium salt of hadacidin in the x-irradiated and cortisone treated rat bearing H.S. 1 tumors clearly attests to the anti-tumor potentials of this compound. Among the 2 routes of administration (intraperitoneal and *per os*) of hadacidin, similar doses of the compound resulted in similar degrees of tumor inhibition. There were 2 tests, one each among the 2 routes of administration which produced considerably lower tumor inhibition than did the other tests. In-

TABLE III. Effects of Hadacidin Administered Intraperitoneally (IP) and Orally (PO) on H. S. 1 in the Rat.

Site	Dose (mg/kg)	Toxicity, avg host wt Δ (g)		Mortality		Avg tumor wt (g)		% inhibition
		Treated	Control	Treated	Control	Treated	Control	
IP	1500, 1x daily	+12	+16	0/18	1/18	4.3	11.1	61*
	3000, 1x "	+12	+11	0/6	0/6	3.2	5.1	37
	4000, 3x "	+22	+20	0/6	0/6	2.6	8.1	68
PO	1500, 1x "	+11	+14	0/6	0/6	3.6	5.7	37
	1600, 3x "	+12	+17	0/6	0/6	3.6	8.9	60
	4000, 3x "	+22	+18	3/42	0/36	2.8	8.4	67*

* Avg of pooled results for 2 or more tests.

spection of Table III clearly shows that each of the cases in question shows a considerably lower mean tumor weight for the controls as compared with the mean weights for the controls in the other tests. This finding is in agreement with observations concerning discrepancies among test results in the embryonated egg with this particular drug. In general, compounds which produce appreciable tumor inhibition also produce considerable host toxicity, however, it is noteworthy that hadacidin produced consistent tumor inhibition without evidence of host toxicity.

Summary. Four transplantable human tumors (H. Ep. 3, H.S. 1, H. Ad. 1 and A-42) were grown on the CAM of chick embryos and used to determine the anti-tumor potential of hadacidin. In testing samples of hadacidin, there was consistent anti-tumor activity. When a comparison among the tumor types of their response to hadacidin was made, A-42 appeared to be consistently more sensitive to hadacidin than were the other types of tumors. Using two different routes of administration, intraperitoneal and *per os*, hadacidin inhibited the growth of H.S. 1 in the x-irradiated and cortisone treated rat. The positive results obtained in the mammalian host are further indications of the anti-tumor potentials of hadacidin.

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