Tumorigenic Effect of 1-Hydrazinophthalazine Hydrochloride in Mice [1]

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ABSTRACT—A solution of 0.125% 1-hydrazinophthalazine hydrochloride, an antihypertensive drug widely used in humans, was given continuously in drinking water for the life-spans of randomly bred Swiss mice. Consumption of the chemical significantly increased the lung tumor incidence from 36 to 60% in females and from 26 to 46% in males, compared to controls. Histopathologically, the tumors were classified as adenomas and adenocarcinomas of the lungs.—J Natl Cancer Inst 61: 1363-1365, 1978.

HPH is a cyclic hydrazine used in medicine as an effective antihypertensive agent (*1*). This drug is believed to act chiefly by sedation of vasopressor centers in the midbrain, but it also has some antiadrenergic action. Adequate doses of the compound decrease arterial blood pressure and peripheral vascular resistance, while increasing heart rate and cardiac output (*2, 3*). It is prescribed clinically in North America to large numbers of people.

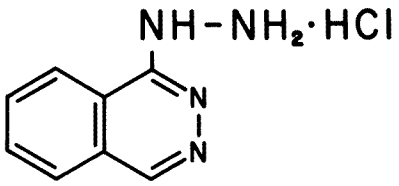
The present investigation is part of an integrated program to reveal the carcinogenic potencies and mode of action of hydrazines. More than 100 hydrazines, hydrazides, and hydrazones are known to exist in the environment as synthetic and naturally occurring compounds. They are used in medicine as drugs and in agriculture as herbicides, and many of them are used as industrial chemicals (*1*). To date, 40 such compounds were shown to induce tumors in laboratory animals (*4-8*).

In this work, the carcinogenicity of HPH administered orally at the maximum tolerated dose for the life-spans of Swiss mice is reported.

MATERIALS AND METHODS

Swiss albino mice from the colony randomly bred by us since 1951 were separated according to sex into groups of 10 and housed in plastic cages with granular cellulose bedding. They were given a Wayne Lab Blox diet in regular pellets (Allied Mills, Inc., Chicago, Ill.) and tap water or the chemical solution ad libitum, as described below.

The chemical used was HPH (hydralazine, Apresoline; mol wt, 196.71; melting point decomposition, 273° C; purity, 93%) obtained from Ciba Pharmaceutical Company, Summit New Jersey. The chemical structure of HPH is shown in text-figure 1.



TEXT-FIGURE 1.—Chemical structure of HPH.

The toxicity study was performed prior to the chronic administration experiment. Five dose levels (wt/vol) of HPH (1, 0.5, 0.25, 0.125, and 0.0625%) were administered in the drinking water for 35 days to Swiss mice. On the basis of four parameters (survival rates, body weights, chemical consumption levels, and histologic changes), the 0.125% dose was found to be suitable for the lifelong treatments. This toxicity technique was developed in this laboratory (*9*).

The solution was prepared three times weekly, and the total consumption of water containing HPH was measured at the same intervals during the treatment period. The solution was contained in brown bottles because of the possible sensitivity of the chemical to light. The 0.125% solution of HPH used for the chronic administration experiment was analyzed by gas chromatography after standing 48 hours at room temperature and was found to contain 92% of the original compound unchanged. The experimental group of the chronic administration study and the controls were as follows.

Group 1.—HPH was dissolved in the drinking water as a 0.125% solution and was given for the life-spans of 50 female and 50 male mice that were 6 weeks (42 days) old at the beginning of the experiment. The average daily consumption of HPH solution per animal was 4.3 ml for the females and 5.9 ml for the males. Therefore, the average daily intake of HPH was 5.4 mg for a female and 7.4 mg for a male.

Group 2.—As untreated controls, 50 female and 50 male mice were kept and observed from weaning time (5 wk of age). Whenever a treated mouse died, a corresponding untreated mouse was killed on the same day.

The experimental and control animals were carefully checked and weighed at weekly intervals, and the gross pathologic changes were recorded. The treated animals were either allowed to die or were killed with ether when found in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered

ABBREVIATION USED: HPH = 1-Hydrazinophthalazine hydrochloride.

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Formalin. Histologic studies were done on the liver, spleen, kidneys, bladder, thyroid gland, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four lobes of the lungs of each mouse, and any other organs showing gross pathologic changes. Sections from these tissues were stained routinely with hematoxylin and eosin, and the abnormalities were studied by light microscopy.

RESULTS

The survival rates of mice after weaning are shown in table 1. The numbers and percentages of animals with tumors and their ages at death (latent periods) are summarized in table 2.

Lung Tumors

Of treated females, 30 (60%) developed 81 tumors of this organ. Of these, 19 mice had 40 adenomas, 2 mice had 2 adenocarcinomas, and 9 mice had 28 adenomas and 11 adenocarcinomas. Their average age at death was 78 weeks; the first tumor was found at the 49th week and the last at the 98th week. In treated males, 23 (46%) developed 58 lung neoplasms. Of these, 14 mice had 21 adenomas, 1 mouse had an adenocarcinoma, and 8 mice had 24 adenomas and 12 adenocarcinomas. Their average age at death was 79 weeks; the first tumor was found at the 60th week and the last at the 96th week of age.

In untreated females, 18 (36%) developed 20 lung tumors. Of these, 15 mice had 17 adenomas and 3 mice had 3 adenocarcinomas. Their average age at death was 78 weeks; the first tumor was found at the 55th week and the last at the 92d week. In untreated males, 13 (26%) developed 16 tumors of this organ. Of these, 9 mice had 9 adenomas, 4 mice had 4 adenocarcinomas, and 1 mouse had 1 adenoma and 2 adenocarcinomas. Their average age at death was 75 weeks; the first tumor was found at the 54th week, and the last at the 95th week of age.

Grossly and microscopically, these tumors were similar to those found and described after various treatments in studies of Swiss mice in this laboratory (*10*).

TABLE 1.—*Treatments and survival rates in HPH-treated and control Swiss mice*

Group	Treatment	Initial No. and sex of mice	No. of survivors, wk of age:									
			10	20	30	40	50	60	70	80	90	100
1	HPH (0.125%) in drinking water, daily for life	50 ♀	50	50	50	50	48	47	36	16	6	—
		50 ♂	50	50	49	49	47	46	35	20	6	—
2	Untreated	50 ♀	50	50	50	50	48	47	36	16	6	—
		50 ♂	50	50	49	49	47	42	31	18	6	—

TABLE 2.—*Tumor distribution in HPH-treated and control Swiss mice*

Group	Treatment	Effective No. and sex of mice	Animals with tumors of:					
			Lungs			Other tissues		
			No. of mice	Percent	Average age at death (range), wk	No. of mice	Type of tumor	Age of animals at death, wk
1	HPH (0.125%) in drinking water, daily for life	50 ♀	30	60	78 (49–98)	7	Malignant lymphomas	60,60,61,63,68,72,91
						1	Pheochromocytoma	86
						1	Adenoma of kidney	64
						1	Angioma in ovary	83
						1	Angioma in liver	71
						1	Fibroma of uterus	75
						1	Adenoma of ovary	77
						1	Adenocarcinoma of ovary	91
						1	Carcinoma of forestomach	84
		50 ♂	23	46	79 (60–96)	5	Angiosarcomas in livers	65,67,89,91,95
						4	Malignant lymphomas	67,77,86,87
						3	Angiomas in livers	78,85,92
						1	Angioma in anal gland	79
						1	Polypoid adenoma of duodenum	81
						1	Adenocarcinoma of cecum	55
						1	Fibroma of nasal cavity	55
2	Untreated control	50 ♀	18	36	78 (55–92)	11	Malignant lymphomas	60,64,69,72,73,74,77,79,88,89,91
						2	Angiosarcomas in livers	78,79
						1	Angioma in ovary	75
						1	Polypoid adenoma and an adenocarcinoma of glandular stomach	88
		50 ♂	13	26	75 (54–95)	5	Malignant lymphomas	56,68,82,88,90
						2	Angiomas in livers	83,90
						2	Fibrosarcomas, subcutaneous	70,76
						1	Sebaceous gland adenoma	83

The typical benign adenomas were nodular and well demarcated from the surrounding tissue; the cuboidal or columnar cells were arranged in regular clusters. The adenocarcinomas exhibited irregular acini formations, often invaded bronchi and blood vessels, and showed many mitoses.

Other Tumors

A few types of tumors other than those of the lung were found in the treated groups (table 2). Because these neoplasms occurred in low incidences, their appearance could not be attributed to the treatment.

DISCUSSION

The current investigation demonstrated for the first time that lung tumors were induced by lifelong administration of 0.125% HPH in the drinking water of randombred Swiss mice, from 6 weeks of age. The incidence of lung neoplasms rose from 36 to 60% in females and from 26 to 46% in males, compared with untreated controls. The statistical analysis by the use of Fisher's exact test (*11*) for 2×2 tables showed that in treated females ($P<0.014$) and males ($P<0.032$), the incidence of lung tumors is significantly higher than in the untreated group. Histopathologically, the lung lesions were classified as adenomas and adenocarcinomas.

HPH is effective in controlling essential hypertension. It is usually administered orally to humans in doses of 1-2 mg/kg body weight. Its effect may be maintained for 3 months or longer by continued administration, but the response tends to decrease. Although it is less effective than are the other hypotensive drugs, it is less likely to produce side effects (*1-3*).

The metabolism of the drug was also studied both in man and in animals. The major metabolite 1-hydrazinophthalazine, which was isolated from the urine of rats and rabbits, was the glucuronide conjugate, which implicates ring hydroxylation as the primary metabolic reaction. The N-acetyl derivative of the compound was also present, and small amounts of the pyruvic acid hydrazone were also found (*12*). The N-acetylation of this drug occurs to a significant extent but appeared to be less important than hydroxylation in the deactivation, inasmuch as almost 50% of the compound is converted to the glucuronide conjugate. This N-acetylation was observed in humans but not in dogs (*13*). Interestingly, approximately 75% of the dose has appeared in the first 24-hour urine collection, with less than 2% of the dose as unchanged drug (*12, 14-16*).

HPH induced statistically significant incidences of tumors in mice in this investigation. This drug is obviously a weak carcinogen and, thus far, probably the least potent of the hydrazine series of compounds tested. In view of the wide-scale exposure of the human population to this hypotensive agent, a risk versus benefit evaluation is certainly needed.

In the present experiment, when a treated animal died, a corresponding animal was killed. This design permitted an accurate comparison and evaluation of tumor incidences and other biologic manifestations of the drug. One commonly used method, i.e., to keep the treated and control animals until spontaneous death, suffers from the unequal survival rates in the 2 groups. An additional time period is thereby provided for the control animals to develop tumors at a time when the treated animals are already dead. The other commonly used technique, i.e., to kill both the treated and control animals at certain stages of the experiment (e.g., 18 or 24 mo of age), is also inadequate. Its main shortcoming is that tumors with long latency periods are excluded from evaluation.

REFERENCES

(*1*) The Merck Index, 9th ed. Rahway, N.J.: Merck, 1976, p 625

(*2*) SOLLMANN T: A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology, 8th ed. Philadelphia: Saunders, 1957, pp 404-405

(*3*) GOODMAN LS, GILMAN A: The Pharmacological Basis of Therapeutics, 5th ed. New York: MacMillan, 1975, pp 705-707

(*4*) TOTH B: Synthetic and naturally occurring hydrazines as possible cancer causative agents. Cancer Res 35:3693-3697, 1976

(*5*) TOTH B, WALLCAVE L, PATIL K, et al: Induction of tumors in mice with the herbicide, succinic acid 2, 2-dimethylhydrazide. Cancer Res 37:3497-3500, 1977

(*6*) TOTH B, NAGEL D: Tumors induced in mice by *N*-methyl-*N*-formylhydrazine of the false morel *Gyromitra esculenta*. J Natl Cancer Inst 60:201-204, 1978

(*7*) TOTH B, NAGEL D, PATIL K, et al: Tumor induction with the *N'*-acetyl derivative of 4-hydroxymethylphenylhydrazine, a metabolite of agaritine of *Agaricus bisporus*. Cancer Res 38: 177-180, 1978

(*8*) TOTH B: Additional series of carcinogenic hydrazines. To be presented at the XII International Cancer Congress, Buenos Aires, Argentina, 1978. In Press

(*9*) ———: A toxicity method with calcium cyclamate for chronic carcinogenesis experiments. Tumori 58:137-142, 1972

(*10*) TOTH B, SHIMIZU H: 1-Carbamyl-2-phenylhydrazine tumorigenesis in Swiss mice. Morphology of lung adenomas. J Natl Cancer Inst 52:241-251, 1974

(*11*) ARMITAGE P: Statistical Methods in Medical Research. London: Blackwell Sci Publ, 1971, pp 135-138

(*12*) MCISSAAC WM, KONDA M: The metabolism of 1-hydrazinophthalazine. J Pharmacol Exp Ther 143:7-13, 1964

(*13*) DOUGLASS CD, HOGAN R: A metabolite of 1-hydrazinophthalazine (hydralazine). Proc Soc Exp Biol Med 100:446-448, 1959

(*14*) BARLOW RB: Actions of peripheral synapses. *In* Introduction to Chemical Pharmacology, 1st ed. New York: Halsted, 1955, pp 249-263

(*15*) PERRY HM JR: A method of quantitating of 1-hydrazinophthalazine in body fluids. J Lab Clin Med 41:566-573, 1953

(*16*) DOUGLASS CD, DILLAHA CJ, DILLAHA J, et al: Inhibition of biologic acetylation by 1-hydrazinophthalazine. J Lab Clin Med 49:561-565, 1957