

Test for Carcinogenicity of Metallic Compounds by the Pulmonary Tumor Response in Strain A Mice¹

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SUMMARY

The production of lung adenomas in strain A mice following multiple i.p. injections of 13 metallic compounds was investigated. A significant increase in the average number of lung tumors per mouse was noted following the administration of lead subacetate, manganous sulfate, molybdenum trioxide, and nickelous acetate. These four compounds can be considered as weakly carcinogenic for lung tumors in strain A mice.

INTRODUCTION

Despite evidence that some metals can produce cancer in man (10), relatively little experimental work has been done in metal carcinogenesis. Several metals have been shown to produce neoplastic responses in animals (6, 20), but these studies need further verification and extension. For example, to the authors' knowledge, there are no data regarding the activity of metals and their position in the periodic table or their modes of carcinogenic action, although some metals are mutagenic (11, 13). In addition, there is little information regarding the carcinogenic activity of metals when administered either as powders, as salts, or as chelates. Further, a comparison of the relative carcinogenicity of various metals in an appropriate test system would provide useful information.

The strain A mouse lung tumor system, first described by Andervont and Shimkin (1), has been applied for the bioassay of numerous chemicals (18). It is based on the principle that carcinogens produce a significant increase in the number of lung tumors in treated animals when compared to controls. The carcinogenic activity of the test compounds can be compared by determining the molar dose of each active compound required to produce an average of 1 lung tumor per mouse (18). In the present study, 13 metallic compounds were examined for their ability to produce lung tumors in strain A mice. The responses obtained are compared to previous data on the activity of these compounds in other bioassay systems.

MATERIALS AND METHODS

Animals. Strain A/Strong male and female mice were purchased from the Strong Research Foundation, San

Diego, Calif. The mice, 6 to 8 weeks old and weighing an average of 18 to 20 g, were randomly distributed among experimental and control groups. They were housed and fed as described previously (19). Hygienic conditions were maintained by weekly disinfection of the animal quarters and twice-weekly changes of the animal cages.

Chemicals. The compounds, all reagent grade and of more than 97 to 99.9% purity, were purchased from the J. T. Baker Chemical Co., Phillipsburg, N. J. Urethan (Matheson, Coleman & Bell, Los Angeles, Calif.) was used as the positive carcinogen in these studies. All chemicals were routinely stored at 4° in the dark.

Solubility tests were conducted using 0.85% NaCl solution and tricapyrin (K & K Laboratories, Plainview, N. Y.). At the doses used, all compounds except chromium sulfate and lead subacetate were soluble in 0.85% NaCl solution. Fresh solutions of the compounds were prepared weekly for injection.

Bioassay. Preliminary toxicology tests were performed as described (19). From these tests, a maximum tolerated dose was determined for each compound. For the bioassays, equal numbers of male and female mice were randomly assorted into groups of 20. When possible, the mice in each group received thrice-weekly i.p. injections of the compound under test for a total of 24 injections. Fewer injections of the more toxic chemicals were given. Three dose levels were used: the maximum tolerated dose, a 1:2, and a 1:5 dilution of the maximum tolerated dose. All mice were killed 30 weeks after the 1st injection, and their lungs were removed and fixed in Tellyesniczky's fluid. After 1 to 2 days, the milky-white nodules on the lungs were counted; a few nodules were examined histopathologically to confirm the typical morphological appearance of the adenoma.

There were 4 control groups: (a) mice receiving 24 i.p. injections of either 0.85% NaCl solution or tricapyrin alone; (b) animals given a single i.p. injection of the positive carcinogen urethan (20 mg/mouse); and (c) untreated mice maintained along with the treated groups to determine the incidence of spontaneous adenoma development during the tests. Experimental and control mice were weighed every 2 weeks during the injection period and at monthly intervals thereafter.

Other organs examined at autopsy for the presence of abnormal lesions were the liver, intestines, thymus, kidney, spleen, salivary, and endocrine glands. Grossly abnormal tissues as well as the lungs were sectioned and examined histologically.

Evaluation of the Data. The frequency of lung tumors in the chemically treated group was compared with that in the

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appropriate vehicle controls by the standard Student's *t* test. Since the vehicle control data were not appreciably different for the 2 sexes, the control data for males and females were combined and compared to mean tumor responses obtained from the treated groups.

RESULTS

Pulmonary Tumors in Controls. Table 1 presents data on the frequency of lung tumors in mice that either received 24 thrice-weekly injections of the 2 vehicles, a single i.p. injection of urethan (20 mg/mouse), or they were untreated.

The incidence of lung tumors in vehicle control and untreated mice is similar to results obtained in previous studies (14, 19). Comparison of the tumor responses in untreated and vehicle control mice indicates that the occurrence of lung tumors was not significantly affected by the injections.

The tumor response to the positive carcinogen, urethan, is nearly identical to that obtained in previous studies (14, 19). These data again demonstrate the stability of the strain A to the induction of lung tumors with chemical carcinogens.

Pulmonary Tumors in Mice Treated with Metallic Compounds. Table 2 summarizes data obtained from bioassays of the 13 metallic compounds. Tricaprylin was used as the vehicle for chromium sulfate and lead subacetate; the remaining compounds were administered in 0.85% NaCl solution. The doses ranged from as low as 7 mg/kg/mouse for cadmium acetate to as high as 4750 mg/kg/mouse for molybdenum trioxide. The results of only 2 doses of cadmium acetate and iron 2,4-pentanedione are given; groups receiving the highest dose of these compounds all died due to delayed toxicity after the injections.

Four of the 13 metallic compounds namely, lead subacetate, manganous sulfate, molybdenum trioxide, and nickelous acetate, produced a significant ($p < 0.05$) increase in the lung tumor response when compared to appropriate controls. There was a well-defined dose-response relationship between the mid- and high doses of these compounds, although the lower doses produced tumor responses similar to the mid-dose. The high dose of cupric acetate produced a mean of 2.0 ± 0.89 tumors per mouse; however, this response was based on only 5 surviving animals. Among the lung tumors observed at autopsy were 7 adenocarcinomas (5 in mice receiving nickelous acetate and 2 in groups receiving vanadium).

On a molar dose basis the most active metallic compound was lead subacetate. A dose of 0.185 mmole/kg was required to produce a mean of 1.47 lung tumors per mouse; assuming a linear dose-response, 0.13 mmole/kg is required for a response of 1 lung tumor per mouse. Therefore, lead subacetate is more than 3 times as active as urethan for which a dose of ~ 0.5 mmole/kg is required for a response of 1 lung tumor per mouse (Table 1). The doses of the other active compounds required for the 1 lung tumor per mouse response are: nickelous acetate, 1.15 mmoles/kg; manganous sulfate, 3.3 mmoles/kg; and molybdenum trioxide, 29 mmoles/kg.

Neoplasms other than lung tumors observed in experimental groups at autopsy included 4 thymomas in mice treated with zinc acetate and 1 salivary gland tumor in a mouse treated with chromium sulfate. The occurrence of these tumors in the various groups was not statistically significant. No tumors other than lung adenomas were observed in the controls.

DISCUSSION

Four of the 13 metallic compounds, namely, nickelous acetate, lead subacetate, manganous sulfate, and molybdenum trioxide, produced a significant increase in the lung tumor response in strain A mice.

The activity of nickelous acetate might have been expected since, as documented in the review articles on metal carcinogenesis by Furst (5), Furst and Haro (6), and Sunderman (20), nickel and compounds of nickel have produced a spectrum of tumors in various animal species, including lung tumors in guinea pigs and rats (9, 12). In addition, nickel is thought to be carcinogenic for the nasal cavity, paranasal sinuses, and lung of man (10). Similarly, lead compounds such as lead phosphate, lead acetate, and basic lead acetate have been shown to induce renal tumors in rodents (3), and lead acetate produced adenomas of the adrenal, thyroid, pituitary, prostate, and lungs following chronic dietary administration to rats (21). In addition, lead chromate was strongly carcinogenic when given i.m. to rats (A. Furst, personal correspondence). However, the life-time administration in the drinking water of either nickel or lead (acetate or oxalate salts) to Swiss mice did not increase the incidence of lung tumors, although there was evidence of the accumulation of both metals in the lungs (16). In the present study, the positive responses to these metallic compounds may have been due to their administration i.p. To our knowledge, there are no data regarding the trans-

Table 1
Lung tumors in vehicle-treated, urethan, and untreated A/Strong mice

Treatment	Duration of experiment (wk)	No. i.p. injections	Survivors/initial	Mice with lung tumors (%)	Av. no. of tumors/mouse
0.85% NaCl solution	30	24	19/20	37	0.42 ± 0.10^a
Tricaprylin	30	24	18/20	44	0.50 ± 0.12
Urethan 20 mg	30	1	18/20	100	21.6 ± 2.81
Untreated	30	0	19/20	31	0.28 ± 0.07

^a Mean \pm S.E.

Table 2
Pulmonary tumors in A/Strong mice treated with metallic compounds

Compound	M.W.	Vehicle ^a	Duration of experiment (wk)	No. of i.p. injections	Total dose (mg/kg of mouse)	No. of animals survivors/initial	Mice with lung tumors	Av. no. of lung tumors/mouse	p
Cadmium(II) acetate	266.53	S	30	23	14	10/20	3	0.40 ± 0.13 ^b	NS ^c
					7	14/20	6	0.71 ± 0.19	NS
Calcium(II) acetate	158.17	S	30	12	1200	19/20	7	0.58 ± 0.13	NS
					600	20/20	3	0.15 ± 0.03	NS
					240	20/20	2	0.15 ± 0.03	NS
Chromium(III) sulfate	392.20	T	30	24	2400	16/20	6	0.63 ± 0.16	NS
					1200	19/20	10	0.79 ± 0.18	NS
					480	20/20	8	0.75 ± 0.17	NS
Cobalt(III) acetate	236.07	S	30	19	475	17/20	10	0.79 ± 0.22	NS
					237	20/20	8	0.70 ± 0.16	NS
					95	20/20	8	0.65 ± 0.14	NS
Cupric(III) acetate	181.63	S	30	24	180	5/20	3	2.00 ± 0.89	NS
					90	18/20	9	0.56 ± 0.13	NS
					36	15/20	4	0.40 ± 0.10	NS
Iron(II) 2,4-pentanedione	254.07	S	30	6	750	9/20	6	1.00 ± 0.36	NS
					375	20/20	10	0.60 ± 0.13	NS
Lead(II) subacetate ^c	807.75	T	30	15	150	15/20	11	1.47 ± 0.38	<0.05
					75	12/20	5	0.50 ± 0.14	NS
					30	17/20	6	0.35 ± 0.09	NS
Manganous(II) sulfate	169.01	S	30	22	660	18/20	12	1.20 ± 0.49	<0.05
					330	20/20	7	0.65 ± 0.15	NS
					132	19/20	7	0.47 ± 0.11	NS
Molybdenum(III) trioxide	143.94	S	30	19	4750	15/20	10	1.13 ± 0.20	<0.05
					2735	19/20	7	0.50 ± 0.13	NS
					950	13/20	4	0.30 ± 0.08	NS
Nickelous(II) acetate	248.86	S	30	24	360	19/20	12	1.26 ± 0.29	<0.01
					180	14/20	7	0.71 ± 0.19	NS
					72	18/20	8	0.67 ± 0.16	NS
Stannous(II) chloride	189.61	S	30	24	1200	4/20	2	0.75 ± 0.38	NS
					600	12/20	5	0.50 ± 0.14	NS
					240	19/20	3	0.16 ± 0.04	NS
Vanadium(III) 2,4-pentanedione	348.27	S	30	24	120	19/20	13	0.79 ± 0.18	NS
					60	12/20	3	0.58 ± 0.12	NS
					24	18/20	4	0.17 ± 0.04	NS
Zinc(II) acetate	219.49	S	30	24	360	16/20	7	0.78 ± 0.22	NS
					180	18/20	8	0.67 ± 0.16	NS
					72	18/20	9	0.67 ± 0.16	NS

^a S, 0.85% NaCl solution; T, tricapylin.

^b Mean ± S.E.

^c NS, not significant.

port, metabolism, and excretion of metals given i.p.

The carcinogenic activity of manganese or molybdenum compounds is not well documented in the literature. However, manganous chloride has been shown to hasten the appearance of lymphosarcoma in mice (4), and both an organomanganese compound and a molybdenum compound were moderately carcinogenic when given i.m. to rats (A. Furst, personal communication). In the present study, manganous sulfate was more active than molybdenum trioxide for lung tumor production in strain A mice.

Lead, manganese, and molybdenum are not listed among the potential metal carcinogens for man (10).

Among the other 9 metals examined in the present study, chromium, cobalt, and iron are listed as potential carcinogens for man (9), and 5 (cadmium, chromium, cobalt, iron, and zinc) in metallic form and/or as salts, have been shown to produce tumors in various species (6, 20). Most experimental tests involved the production of sarcomas at the injection site. However, zinc salts induced tumors only when injected into testes of fowl (2), and not by s.c. (7) or

i.m. (8) routes. All 5 metallic compounds were negative for lung tumor production in strain A mice. Although chromium sulfate was administered at high doses, the inactivity of cobalt acetate, iron(II) derivative, zinc acetate, and especially cadmium acetate may have been related to their toxicity at relatively low doses. Similarly, Schroeder (17) found that cadmium acetate was very toxic and chromic acetate nontoxic when administered in the drinking water to Swiss mice. Also, in agreement with the results of the present study, Schroeder *et al.* (15, 16) reported that the life-time administration of cadmium and chromium (acetate or oxalate salts), stannous chloride, and vanadyl sulfate of Swiss mice did not increase the incidence of lung adenomas.

To the authors' knowledge, calcium, copper, vanadium, and tin compounds have not been tumorigenic in any test system. They were also negative for lung tumor production in strain A mice at the doses administered.

The mechanism(s) by which metals produce tumors has yet to be determined. There is some evidence that carcinogenic metals are mutagenic; e.g., they have been shown to produce chromosomal aberrations in cultured human diploid cells (13) and to be mutagenic for recombinant-deficient strains of *Bacillus subtilis* (11). However, in the latter study there was not complete correlation between carcinogenicity and mutagenicity. For example, salts of the more weakly active metals such as manganese and molybdenum were mutagenic; whereas those of the more active metals such as lead, cobalt, iron, zinc, and nickel were not mutagenic. Clearly, studies aimed at elucidating the mode(s) of action of carcinogenic metals are required for our further understanding of metal carcinogenesis.

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