

Inhibition by Magnesium and Calcium Acetates of Lead Subacetate- and Nickel Acetate-induced Lung Tumors in Strain A Mice¹

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ABSTRACT

The ability of the physiologically essential divalent metals calcium and magnesium to inhibit the tumorigenic activities of lead and nickel towards the lungs of strain A mice was investigated. The tumorigenic salts lead(II) subacetate and nickel(II) acetate were injected i.p. at their maximal tolerated doses (0.04 mmol/kg/injection of each metal) for a total of 24 injections, whenever possible. Calcium(II) acetate and magnesium(II) acetate were administered in the same preparation along with the lead and nickel salts at molar doses of approximately 1, 3, 10, and 30 times the maximal tolerated dose of the tumorigen. The animals were sacrificed 30 weeks after the first injection, and the lung tumors were counted. The lead and nickel salts, administered alone, each produced a significant increase in the observed number of lung adenomas per mouse. When administered with any of the doses of calcium acetate or magnesium acetate tested, neither lead subacetate nor nickel acetate showed any significant tumorigenic activity. Calcium acetate alone (total dose, 11 mmol/kg of body weight) appeared to yield a significant rise in lung adenomas observed. The results indicate an antagonism between magnesium and calcium and the tumorigenic metals nickel and lead.

INTRODUCTION

The carcinogenic activity of some divalent metals has been recognized since at least the early 1930s (9). The list of carcinogenic divalent metals exhibiting a stable divalent state includes beryllium (7), titanium (3), manganese (20), cobalt (22), nickel (9, 10, 23, 24), cadmium (8), and lead (6). These metals share with the physiologically essential divalent metal cations magnesium and calcium the property that their valence electrons occupy an external s orbital. As such, the divalent metal carcinogens exhibit many of the chemical and physiological properties of magnesium and calcium. In fact, many of the above metal carcinogens have exhibited effects that are antagonistic to magnesium(II) or calcium(II) in one or more biological systems. The physiological competition of lead(II) versus calcium(II) and of magnesium(II) and calcium(II) versus cadmium(II) has been especially well studied (2, 5, 11, 16-18, 25-27). Finally, magnesium deficiency has been shown to produce lymphomas and thymomas in rats (1, 12).

Previous studies have shown that the metal carcinogens nickel(II) and lead(II) produce adenomas in the lungs of strain A mice (20). These observations extend to the lungs of mice

previous findings of the carcinogenic activity of lead salts toward the kidneys of rats and the versatile carcinogenicity of nickel and its compounds in rodents (9, 10). As a bioassay for chemical carcinogens, lung adenoma formation offers the advantages of ease of performance and relative rapidity. This system was thus selected to study the possible inhibitory effects of calcium(II) and magnesium(II) salts on the tumorigenicity of lead(II) and nickel(II).

MATERIALS AND METHODS

Nickel(II) acetate, lead(II) subacetate, calcium acetate, and magnesium acetate (reagent grade) were obtained from J. T. Baker Chemical Company (Phillipsburg, NJ). Tricaprylin, which was used as a vehicle for injection of lead(II) subacetate, was obtained from Sigma Chemical Company (St. Louis, MO). Equal numbers of male and female A/St mice (L. C. Strong Research Foundation, Inc., San Diego, CA), 6 to 8 weeks of age, were used as test animals.

Doses of nickel(II) acetate (10.7 mg/kg of body weight/injection [$\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$; *M*, 248.86] and lead(II) subacetate (10.0 mg/kg of body weight/injection [$\text{Pb}_2(\text{CH}_3\text{COO})_2(\text{OH})_4$; *M*, 807.75] were used throughout these experiments to induce lung tumors. Each injection thus contained the tumorigenic metal (0.04 mmol/kg of body weight) and was administered i.p. These doses had been shown to constitute the maximal tolerated dose in these animals and to be tumorigenic toward the lungs of mice (20).

To assess the effect of calcium on nickel-induced lung tumorigenesis, mixtures of nickel(II) acetate and calcium acetate were prepared in approximately 1:1, 1:3, 1:10, and 1:30 molar ratios in 0.9% NaCl solution, and each mixture was injected 3 times weekly into groups of 30 mice, for a total of 24 injections. One group of mice was given injections of nickel(II) acetate alone 24 times i.p., and one group was given injections of calcium acetate [$\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$; *M*, 176.19] alone 24 times i.p. at a dose equivalent to the amount of calcium acetate present in the 1:10 mixture (total dose, 11.0 mmol/kg). One group of mice was given injections of 0.2 ml of the 0.9% NaCl solution vehicle 24 times i.p. To assess the effect of calcium on lead-induced lung tumorigenesis, mixtures of lead(II) subacetate and calcium acetate were suspended in 1:1, 1:3, and 1:10 molar ratios in tricaprylin, and each mixture was injected 3 times weekly into groups of 30 mice for a total of 20 injections. One group of mice was given injections of lead(II) subacetate alone 20 times i.p. One group was given injections of calcium acetate alone 20 times with a total of 9.2 mmol/kg i.p. One group of mice was given injections of 0.1 ml of the tricaprylin vehicle 20 times i.p.

To assess the effect of magnesium on lung tumorigenesis by nickel, mixtures of nickel(II) acetate and magnesium acetate [$\text{Mg}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$; *M*, 214.46] were prepared in approximately 1:1, 1:3, 1:10, and 1:30 molar ratios in 0.9% NaCl solution; each mixture was injected 3 times weekly into groups of 30 mice, for a total of 24 injections. The group of mice given injections of nickel(II) acetate alone and the 0.9% NaCl solution vehicle-treated group from the calcium experiment described above were utilized in this magnesium experiment. One group of mice was given i.p. injections of magnesium acetate 24 times at a dose equivalent to the amount of magnesium acetate present in the 1:10 mixture (total dose, 11.0 mmol/kg). To assess the effect of magnesium on lead-induced lung tumorigenesis, finely powdered mixtures of lead(II)

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subacetate and magnesium acetate were suspended in 1:1, 1:3, and 1:10 molar ratios of the 2 metals in tricapylin. Because of the elevated toxicity of the lead:magnesium combinations, each mixture was injected i.p. 3 times weekly into groups of 30 mice, for a total of only 9 injections. As controls, one group of mice was given i.p. injections of lead(II) subacetate alone 9 times, and one group was given i.p. injections of a total of 4.1 mmol of magnesium acetate alone 9 times. One group of mice was given i.p. injections of 0.2 ml of tricapylin vehicle 9 times. No evidence of coprecipitation or of any other reaction could be observed with any of the combinations of metal salts used in these studies.

Thirty weeks after the first injection, all mice were sacrificed, and the lungs were placed in Tellyesniczky's fluid (100 ml of 70% ethanol:5 ml of formalin:5 ml of glacial acetic acid). The lungs were then examined under a dissecting microscope (x10), and the surface adenomas were counted. A few surface nodules were examined histologically to confirm the typical morphological appearance of the adenoma. The average number of tumors per lung in the groups given injections of nickel(II) acetate, lead(II) subacetate, calcium acetate, or magnesium acetate alone were statistically compared with the appropriate vehicle-treated control group by Student's *t* test. The average number of tumors per lung in the mouse groups given injections of the mixtures was compared statistically with the average number of tumors per lung in the mouse groups treated with the corresponding tumorigenic metal by Student's *t* test.

RESULTS

Both tumorigenic metal salts, nickel(II) acetate and lead(II) subacetate (Tables 1 and 2), produced a statistically significant lung tumor response under the exposure conditions used in these experiments. Calcium acetate, when administered alone (total dose, 11.0 mmol/kg), also produced a lung tumor response (Table 1). This lung tumor response was statistically significant when calcium acetate was administered in a 0.9% NaCl solution vehicle. Magnesium acetate alone did not produce a significant lung tumor response (Table 2). When exposure to tumorigenic doses of nickel(II) acetate and lead(II) subacetate was coupled with simultaneous exposure to calcium acetate, the lung tumor response to these tumorigenic metals was completely inhibited (Table 1). This inhibition was evident at all molar ratios of tumor-

Table 1

Effect of calcium on the pulmonary adenoma response to metal tumorigens

Tumorigenic metal salt	Tumorigenic metal: calcium acetate molar ratio ^a	No. of i.p. injections	Survivors/initial	Av. no. of lung tumors/mouse
None (0.9% NaCl solution)		24	25/30	0.32 ± 0.12 ^b
None (0.9% NaCl solution)	0:10	24	26/30	1.04 ± 0.26 ^c
Nickel(II) acetate ^d	1:0	24	24/30	1.50 ± 0.46 ^c
	1:1	24	29/30	0.40 ± 0.12 ^e
	1:3	24	30/30	0.20 ± 0.12 ^e
	1:10	24	30/30	0.27 ± 0.13 ^e
	1:30	24	15/30	0.0 ± 0.0 ^e
None (tricapylin)		20	26/30	0.35 ± 0.09
None (tricapylin)	0:10	20	26/30	0.62 ± 0.17
Lead(II) subacetate ^f	1:0	20	20/30	0.86 ± 0.20 ^c
	1:1	20	23/30	0.21 ± 0.10 ^e
	1:3	20	18/30	0.22 ± 0.10 ^e
	1:10	20	16/30	0.13 ± 0.09 ^e

^a Calcium acetate mixed with tumorigenic metal in the appropriate vehicle in the molar ratio indicated; this mixture was injected i.p. for either 24 injections (nickel) or 20 injections (lead).

^b Mean ± S.E.

^c Significantly above vehicle-injected control group (*p* < 0.05).

^d Nickel (0.04 mmol/kg/injection i.p.) in 0.1 ml of 0.9% NaCl solution.

^e Significantly below group treated with tumorigenic metal only (*p* < 0.05).

^f Lead (0.04 mmol/kg/injection i.p.) in 0.1 ml of tricapylin.

Table 2

Effect of magnesium on the pulmonary adenoma response to metal tumorigens

Tumorigenic metal salt	Tumorigenic Metal: magnesium acetate molar ratio ^a	No. of i.p. injections	Survivors/initial	Av. no. of lung tumors/mouse
None (0.9% NaCl solution)		24	25/30	0.32 ± 0.12 ^b
None (0.9% NaCl solution)	0:10	24	29/30	0.59 ± 0.16
Nickel(II) acetate ^c	1:0	24	24/30	1.50 ± 0.46 ^d
	1:1	24	29/30	0.34 ± 0.10 ^e
	1:3	24	29/30	0.28 ± 0.10 ^e
	1:10	24	27/30	0.19 ± 0.05 ^e
	1:30	24	29/30	0.21 ± 0.08 ^e
None (tricapylin)		9	21/30	0.29 ± 0.11
None (tricapylin)	0:10	9	29/30	0.45 ± 0.09
Lead(II) subacetate ^f	1:0	9	20/30	1.65 ± 0.36 ^d
	1:1	9	1/30	
	1:3	9	13/30	0.08 ± 0.08 ^e
	1:10	9	18/30	0.28 ± 0.11 ^e

^a Magnesium acetate mixed with tumorigenic metal in the appropriate vehicle in the molar ratio indicated; this mixture was injected i.p. for either 24 injections (nickel) or 9 injections (lead).

^b Mean ± S.E.

^c Nickel (0.04 mmol/kg/injection i.p.) in 0.1 ml of 0.9% NaCl solution.

^d Significantly above vehicle-injected control group (*p* < 0.05).

^e Significantly below group treated with tumorigenic metal only (*p* < 0.05).

^f Lead (0.04 mmol/kg/injection i.p.) in 0.2 ml of tricapylin for a total of 9 injections.

igenic metal:calcium acetate which were investigated.

When treatment with nickel(II) acetate and lead(II) subacetate occurred simultaneously with that of magnesium acetate, the lung tumor response was again completely inhibited (Table 2). This inhibition occurred at all molar ratios of tumorigenic metal:magnesium acetate. It is interesting to note that an equimolar mixture of lead(II) subacetate and magnesium acetate was very toxic to strain A mice, and this toxicity decreased as the ratio of magnesium acetate:lead(II) subacetate in the mixture increased (Table 2).

DISCUSSION

The present study demonstrates that the physiologically essential divalent cations magnesium and calcium prevent the rise in lung adenoma formation seen in Strain A mice treated with nickel acetate and lead subacetate. Such results provide evidence that the carcinogenic activity of divalent metals may occur, in part, through an antagonism with divalent magnesium and/or calcium. Consistent with this proposal are the observations that magnesium deficiency produces thymomas in rats (1, 12) and that tumors of the bone, a tissue with a very high requirement for magnesium and calcium, can be produced by the divalent metal carcinogens beryllium (7) and nickel (9). Biochemical antagonisms between divalent metal carcinogens and physiologically essential divalent metal cations have been described previously (16-18, 25-27). Thus, lead(II) competes with calcium(II) (17) and magnesium(II) (2) for intestinal absorption. Cadmium(II) competes similarly with calcium(II) for absorption (21, 26), and cadmium(II) toxicity is inhibited by zinc(II) and calcium(II) (5, 11, 25). There is thus considerable biochemical evidence rendering plausible an antagonism hypothesis of metal carcinogenesis. Jennette (13) has recently written a review suggesting metal antagonism to explain heavy metal toxicity. Sunderman *et al.* (24) have shown that manganese(II) inhibits the sarcomagenic activity of nickel(II) subsulfide.

The ubiquitous roles of calcium(II) and magnesium(II) in cellular metabolism make speculation on the prospective molecular site responsible for the antagonism of metal carcinogenesis simple but verification difficult. Divalent metal carcinogens have been shown to penetrate to many of the sites of normal magnesium and calcium function. Thus, nickel bound to DNA has been isolated from the livers and kidneys of nickel-treated rats (4). Several divalent metal carcinogens induce infidelity of DNA synthesis *in vitro* (19). Divalent metal carcinogens often appear to share calcium transport systems (16, 21, 26). The great biological importance of calmodulin would seem to make it a reasonable target for future investigations on prospective mechanisms of divalent metal carcinogenesis (15).

The elevated incidence of lung adenomas noted in the present study in one group of mice treated with a high dose of calcium acetate is difficult to interpret. This effect was seen only in mice receiving a total dose of calcium (11.0 mmol/kg administered in 0.9% NaCl solution (Table 1). Mice treated with a lower dose of calcium(II) in tricaprilyn or with either dose of magnesium(II) developed lung adenoma incidences ranging from 55 to 85% above those seen in the corresponding control mice; these increases were not found to be statistically significant. However, such results do raise the possibility that even the essential divalent metals at high concentrations may be tumorigenic. The infidelity of DNA synthesis induced by calcium(II) *in vitro* is consistent with this proposal (19). If calcium(II) and magnesium(II) are definitively demonstrated to be tumorigenic, then the present studies would indicate that this activity is inhibited by nickel(II) and lead(II). In the present investigation, magnesium acetate exhibited somewhat anomalous effects on lead subacetate toxicity; the animals receiving a 1:1 molar ratio of lead(II):magnesium(II) survived far less well than did those treated with the 1:0, 1:3, and 1:10 molar ratios. The antagonism between the physiological divalent metal cations and their carcinogenic homologues may thus be quite complex. It may involve physicochemical interactions at the injection site, shared transport mechanisms, or biochemical competition at critical macromolecular binding sites. Recent studies from this laboratory have shown that the inhibitory effects of magnesium and calcium acetates on lung tumorigenesis in strain A mice could not be ascribed to the prevention by calcium(II) and magnesium(II) of total nickel(II) accumulation in the target organ (14). Further investigations on the metabolic interactions of nickel(II) and lead(II) with calcium(II) and magnesium(II) are required to elucidate the mechanisms underlying the antagonistic effects of these metals.

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