Effect of Lead on Tissue Disposition of Nitrilotriacetic Acid (NTA) in Rats

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Nitrilotriacetic acid (NTA) is a good sequestering agent and is being used as a partial replacement for phosphates in certain household detergent formulations. NIXON et al. (1972) and MICHAEL and WAKIM (1971) reported the toxicity of NTA in rats. NOLEN et al. (1972) showed that orally administered NTA would decrease tissue concentrations of cadmium but had no detectable effect on tissue concentrations of methylmercury in rats. Little information is available on the effect of lead on the tissue disposition of NTA in rats. The present studies were part of our general investigations into the toxicities of NTA in mammals, and were designed to study the tissue disposition of NTA in rats previously treated with lead (as lead acetate).

Materials and Methods

Nitrilotriacetic acid (carboxyl ^{14}C, specific activity 57 mCi/mmol) was purchased from Amersham Searle. Radiochemical purity was found by thin-layer chromatography (TLC) to be greater than 98%. This compound was diluted with unlabelled NTA (Aldrich Chemical) to give 3.36 μCi/mg, and was used in the experiments. A solution of NTA containing 15.2 mg NTA-1-^{14}C/ml (51 μCi) was prepared and adjusted to pH 7.2 with 1 N sodium hydroxide. A fresh lead acetate solution (2 mg lead/ml) was prepared every second day with freshly boiled distilled water. Twelve male Sprague Dawley rats weighing approximately 220-240 g were randomly divided into a test and a control group. The test animals received lead acetate solution as their only source of drinking water while the control animals were given tap water, and both groups had access to food ad libitum for thirteen days. Twenty-four hours prior to NTA administration all animals were deprived of food and the test animals were given tap water instead of the lead acetate solution. All animals were intubated with NTA-1-^{14}C solution (68.2 mg/kg, 51 μCi/kg)and were housed individually in metabolism cages with food and water ad libitum. Urine and feces were collected at the sacrifice times. Two rats from each group were anesthetized with ether and exsanguinated at 6, 24 and 72 hours after administration. The brain, muscle, adipose, heart, lung, liver, spleen, skin, kidney, a piece of thigh bone and testes were removed, freeze-dried, and pulverized. The powdered tissue (60 mg) and feces (60 mg) were rehydrated with water (0.3 ml) and digested with Soluene-100 (1.2 ml, Packard) at 50°C overnight. The digests were dissolved

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in Dimulune-30 (10 ml, Packard) and radioactivity determined for their radioactivity on a Mark III Liquid Scintillation System (Amersham Searle). Quench was corrected by use of an external standard. Urine (50 µl) was dissolved in Aquasol (10 ml, New England Nuclear) and counted directly. The combined urine was evaporated to dryness. The residue was extracted with ethanol (50 ml) and the extract was chromatographed on a silica gel plate (250 µ, Analtech) in a solvent system of n-butanol:acetic acid: water (60: 15: 25). The band containing radioactive material as detected by Actigraph III (Nuclear Chicago) was removed from the plate and extracted with ethanol (30 ml). Dried feces were extracted with ethanol (50 ml) and the concentrated extract was chromatographed in a manner similar to that described for the treatment of urine. The ethanolic extracts were methylated with diazomethane (Diazald, Aldrich) and examined at 70 eV by gas chromatograph-mass spectrometer (GC-MS) (Varian Mat III) equipped with a 6' x 1/8" id glass column packed with 3% OV-17 on 80-100 mesh Chromosorb WHP, and a flame ionization detector.

Results and Discussion

The tissue distribution of NTA-1-^{14}C in rats is given in TABLE 1. The highest level was found in the kidney of both control and lead-treated rats, followed by the intestine, bone, liver, pancreas and lung. The skin, bladder, spleen, heart, testes, brain, adipose and muscle also had some radioactivity. It was observed that the levels in all tissues were comparatively lower for the lead-treated rats than for the control. The tissue contents of NTA rapidly declined for both control and lead-treated rats. After 72 hours the thigh bone of control rats retained the highest level of NTA. But this was not found to be the case with the lead-treated rats. These results appear to indicate that lead facilitated elimination of NTA from the tissue presumably as NTA-lead chelates. These results were also consistent with those found by MICHAEL and WAKIM (1971) who reported that the skeleton retained highest concentration of NTA 72 hours after oral administration to rats. MAHAFFEY and GOYER (1972) showed that Na-NTA decreased the lead content of rat tissues, and hence did not increase the pathological effects of lead. It was not likely that lead would increase the toxicity of NTA because NTA-lead chelate was rapidly removed from tissues.

When the tissue levels were expressed as per cent of the total administered material, they only accounted for less than 5% in both control and lead-treated rats. Thus, it was not surprising that no great difference in rate of excretion can be observed between the two groups of animals. More than 80% of NTA was excreted within six hours for both the groups. Of these approximately 60% was eliminated via the urine and 20% via feces.

TABLE I

Tissue distribution of NTA-1-^{14}C in (A) control rats, (B) rats receiving lead acetate solution (2 mg lead/ml) as their drinking water for 13 days. Data represents the average value of two rats, and are expressed as DPM/mg dry tissue. The variations of duplicate determinations were found to be within 10%.

	6 h		24 h		72 h	
	A	B	A	B	A	B
Kidney	3263	1421	475	271	176	65
Intestine	1340	702	148	100	63	24
Liver	379	211	125	60	82	33
Bone	625	243	247	105	183	29
Pancreas	340	215	53	31	45	20
Bladder	122	287	71	48	20	27
Spleen	116	104	92	61	48	26
Lung	211	132	100	57	46	26
Heart	61	49	38	30	26	19
Testes	92	63	55	36	40	17
Skin	106	44	57	22	39	19
Brain	24	15	14	7	11	4
Adipose	99	11	43	17	18	5
Muscle	49	23	24	12	22	11

The only radioactive compound associated with urine and feces was found to be unchanged NTA. Radioactivity in urinary and fecal extracts migrated on TLC as a single peak with an Rf value of 0.88 comparable to authentic NTA. GC-MS analysis of the methylated extract showed a weak but significant molecular ion at m/e 233 ($C_9H_{15}NO_6$), characteristic of NTA-trimethyl ester.

These preliminary results revealed that the presence of lead in the tissue facilitated elimination of NTA. Further work is still in progress to investigate interactions of NTA with lead, and will be reported elsewhere.

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