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Accumulation of Lead in Tissues After Its Administration in Drinking Water to Laboratory Rats

O. MESTEK[1], Z. DEYL[2], I. MIKŠÍK[2], J. NOVOTNÁ[3], I. PFEIFER[4], J. HERGET[5]

[1]Department of Analytical Chemistry, Institute of Chemical Technology, [2]Institute of Physiology, Academy of Sciences of the Czech Republic, [3]Department of Medical Chemistry and Biochemistry, Second Faculty of Medicine, Charles University, [4]Institute of Preventive Medicine, Second Faculty of Medicine, Charles University and [5]Department of Physiology, Second Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

Lead administered to laboratory rats in drinking water (0.1–0.8 %) as lead acetate solution tends to accumulate in collagen-rich tissues such as tendons and the skin. The amount of lead deposited (and also zinc present in the tissue without its supplementation) correlates with the blood supply to the tissue investigated. The highest deposits of lead were observed in placenta and chorionic membranes, though here only about 60% are collagen-bound. No differences in the drinking habits of the animals were observed and also at lower concentrations of lead in the drinking water no dose dependence was revealed. However, at 0.8 % of lead in drinking water considerable accumulation of lead was observed in all tissues investigated.

Key words
Lead – Collagen – Placenta and chorionic membranes

Introduction

Although lead does not have a distinct function in the human body, its toxicity has been known for many years (Goyer and Mahaffey 1972, for review see Veromysse 1982). The toxic effect of lead varies considerably from gross morphological changes to more subtle biochemical alterations when the toxic element is present in lower concentrations. Lead, like other heavy metals, can be accumulated in the body. In young children it shows a predilection for the central nervous system, haematopoietic tissue and kidneys (Pentschen and Garro 1966).

In general, the uptake of lead from various sources suggests a three compartmental tool for lead metabolism, namely blood lead, lead accumulated in soft tissues (collagen and keratin being the target proteins) and skeleton where it appears to compete with calcium for the binding sites (for review see Grandjean and Olsen 1984).

In the sixties, it was demonstrated by Adam *et al.* (1965, 1968) that gastrointestinal absorption or intramuscular administration of heavy metals results in connective tissue deposits that can be revealed by the cross-striation pattern of collagen fibres in the electron microscope. It was also emphasized that this cross striation pattern is virtually the same as seen with the administration of gold complexes *in vivo* or uranyl acetate staining of collagen fibres *in vitro*. It was hence concluded that the side-chains of acidic amino acids of the collagen polypeptide chains are likely to be involved in this interaction.

Fax +420 2 293 894
E-mail physres@sun1.biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

As far as lead toxicity is concerned, there are numerous target points about which one can speculate; the various aspects have been recently surveyed by Lansdown (1995). There are several types of tissues that can be sampled for lead in the vertebrate body; blood lead may be a useful index of lead consumed or inhaled through occupational exposure. However, it cannot take into account the amounts of lead deposited in bone nor does it reflect accurately the amount of lead secreted in the hair shaft. The damage to the body caused by lead intake can be both direct, owing to its deposition in tissues and indirect, owing to metabolic disturbances (e.g. by interfering with selected metabolic pathways such as the inhibition of δ-aminolevulinic acid dehydratase in haeme biosynthesis) (Hudak *et al.* 1992). It may seem surprising that to our knowledge only very limited information is available so far about lead deposits in soft connective tissue (Mykkanen *et al.* 1979). The only fact that seems to be accepted, though not proven, is that there is nothing like a lead saturation point in any tissue; surprisingly enough no direct evidence for this is yet available (Lansdown 1995). On the other hand, pronounced dose-dependent toxic effects of lead administration were reported in early ontogeny (Mykkanen *et al.* 1979).

In the present communication, we attempted to determine lead accumulation in three types of collagen possessing tissues, namely the tail tendon, skin and placenta and chorionic membranes with the aim to reveal whether or not the lead deposits in collagen are bound strongly enough to survive association of the protein and if so, which domains of the collagen molecule are affected by lead deposit formation.

Table 1. Optimum ICP-MS operating conditions

R.f. power	1000 W
Dwell time	50 ms
Sweeps/replicate	20
No. of replicates	4
Total acquisition time per a.m.u.	4 s
Acquisition mode	peak hopping
Ar nebulizer flow	0.80 $l.min^{-1}$
Ar plasma flow	17 $l.min^{-1}$
Ar auxiliary flow	1.2 $l.min^{-1}$
Lens voltage	autolens mode, optimized before each measurement
Sample uptake rate	1 $ml.min^{-1}$

Methods

Animals and tissues

Female Wistar rats, purchased from a local dealer, 240±30 g body mass were used in the experiments. Lead was administered as lead acetate (0.1, 0.2 and 0.8 % in drinking water) in appropriate concentrations. The control group received tap water. The amount of water consumed did not differ between the groups. After 6 weeks on the lead acetate drinking regime the animals were made pregnant. At the end of pregnancy, they were killed under ether anaesthesia and samples of the skin, tendon and placenta together with chorionic membranes were removed. The skin was freed from fat, hair and muscle, cut into small pieces and lyophilized. Appropriate aliquots were taken for ICP-MS analysis; the tail tendons were lyophilized directly, placenta with chorionic membranes were washed with double distilled water to remove residual blood and were lyophilized; dry mass and hydroxyproline content of all samples was estimated (Deyl *et al.* 1978).

Aliquots of the direct tissue (0.5 g) were extracted with 6 M urea (5 ml) to remove the main portion of non-collagenous proteins and the insoluble residue was dialyzed against one liter of double distilled water overnight (twice) with stirring. The insoluble residue was spun off, lyophilized and taken for ICP-MS analysis.

Determination of lead and zinc by inductively coupled plasma mass spectrometry (ICP-MS)

Both urea-untreated and urea-treated samples were assayed for.

Instrumentation

Measurements were performed on Perkin Elmer Elan 6000 (Perkin Elmer, Norwalk, CT, USA), sample decomposition was accomplished in a microwave mineralizer with a focused field BM-1S/II (Plazmotronika, Wroclaw, Poland).

Reagents and standard

Lead and zinc standard solutions 1000 mg.l^{-1} (Analytika, Prague, Czech Republic) were used for preparation of the working standard. Nitric acid and hydrogen peroxide used for sample decomposition were of Suprapur^{R} grade (Merck, Darmstadt, Germany). Deionized Mili-Q water (Millipore, Bedford, MA, USA) was used for preparation of all solutions.

Procedure

Dry samples (30–100 mg) were decomposed with concentrated HNO_3 (1 ml) and hydrogen peroxide (1 ml) in a microwave mineralizer for 10 min. The resulting solution was transferred into a 25 ml volumetric flask and after addition of 0.5 ml solution containing 5 mg.l^{-1} In and 5 mg.l^{-1} Bi was diluted to volume. One reagent blank per each six samples was prepared by the same way. Two calibration standards containing 100–1000 μg.l^{-1} Zn and 4–40 μg.l^{-1} Pb in 2 % HNO_3 together with blank solution were prepared. The measurements were carried out on a PE Elan 6000 instrument applying conditions shown in Table 1. The signal intensity of ^{66}Zn together with ^{115}In as internal standard was measured and no mathematical corrections for interferences were made during measurements. Lead was quantified as a sum of signals of ^{206}Pb, ^{207}Pb and ^{208}Pb isotopes and ^{209}Bi was used as internal standard.

Table 2. Lead and zinc content of selected rat tissues after lead acetate administration

Tissue	Pb acetate content in drinking water (%)	Pb (μg/g collagen)	Zn (μg/g collagen)
Skin	0.0	ND	5.98±0.96
	0.1	2.43±0.41	6.56±1.21
	0.2^{+}	2.36±0.62	9.46±3.72
	0.2	2.75±0.83	5.74±1.87
	0.8	5.78±0.45	9.18±2.96
	0.8*	2.39±0.58	7.07±2.03
Tail tendon	0.0	ND	31.78±6.92
	0.1	0.76±0.63	26.38±5.32
	0.2+	0.83±0.27	21.85±2.97
	0.2	0.89±0.26	19.84±4.95
	0.8	7.31±0.32	15.23±3.97
	0.8*	2.65±0.27	23.71±4.76
Placenta and chorionic membranes			
	0.0	ND	513.11±26.94
	0.1	129.49±11.70	542.27±36.82
	0.2^{+}	146.43±20.60	603.62±48.20
	0.2	123.84±17.91	495.15±20.38
	0.8	1027.88±79.39	687.73±82.30
	0.8*	650.25±29.34	553.32±75.34

*Numbers represent means ± S.D. from six assays except the group that was administered EDTA and lead simultaneously, where only three animals were available at the end of the experiment (ND stands for non-detected). * Group was given 0.8 % Pb + EDTA simultaneously, $^{+}$ Group was given 0.2 Pb + 0.2 % $ZnSO_4$ simultaneously*

Results and Discussion

As is shown in Tables 2 and 3, laboratory rats being administered lead acetate in drinking water accumulate lead in collagen-rich tissues such as the skin and tail tendon. At low doses (0.1 or 0.2 %) of lead acetate in the drinking fluid, there is virtually no dose-dependent accumulation. The taste of water

supplemented with the lead salt obviously does not influence drinking, as no differences in water consumption were observed with respect to the presence or absence of the lead salt (data not shown). However, at the dose 0.8% of lead acetate, a considerable increase in lead accumulation was found in the investigated tissues. On the other hand, while previous administration of heavy metals (i.p. injection) as EDTA (or other types) of lead complexes increased the uptake of these metals by the connective tissue, this effect was not observed when EDTA was added to the drinking water. It may be speculated that the stability constant of the Pb-EDTA complex is higher than that of the Pb-connective tissue proteins and, consequently the administration of higher doses of Pb-EDTA complex in drinking water is ineffective in terms of Pb accumulation.

Table 3. Lead and tissue content of selected rat tissues after treatment in 6 M urea

Tissue	Pb acetate content in drinking water (%)	Pb (μg/g collagen)	Zn (μg/g collagen)
Skin	0.0	ND	5.98±1.96 (100 %)
	0.1	1.96±0.31 (80 %)	6.29±1.67 (95 %)
	0.2+	2.01±0.67 (85 %)	8.34±2.12 (88 %)
	0.2	1.87±0.32 (68 %)	4.25±0.97 (74 %)
	0.8	4.77±0.44 (82 %)	10.73±1.98 (116 %)
	0.8*	1.97±0.28 (82 %)	6.87±2.05 (97 %)
Tail tendon	0.0	ND	19.29±2.01 (60 %)
	0.1	0.76±0.17 (100 %)	22.37±1.98 (84 %)
	0.2+	0.82±0.22 (98 %)	20.87±2.36 (95 %)
	0.2	0.85±0.23 (95 %)	17.46±2.04 (88 %)
	0.8	7.03±0.71 (96 %)	14.29±1.65 (93 %)
	0.8*	2.55±0.20 (96 %)	22.77±1.23 (96 %)
Placenta and chorionic membranes			
	0.0	ND	348.15±26.15 (67 %)
	0.1	65.29±10.20 (50 %)	368.29±37.28 (68 %)
	0.2+	71.42±14.50 (48 %)	492.73±36.15 (81 %)
	0.2	58.38±13.70 (47 %)	335.68±21.29 (67 %)
	0.8	596.56±75.20 (58 %)	551.45±42.27 (80 %)
	0.8*	322.71±38.30 (49 %)	427.32±77.27 (77 %)

Numbers in parentheses represent recovery (compare with Table 2). For other legend see Table 2.

The doses 0.1–0.2 % of the lead salt caused lead accumulation in the skin to almost threefold values (2.75 μg/g) compared to the tail tendon (0.89 μg/g); on the other hand, at the high dose (0.8 %) of lead acetate, the accumulation of lead was higher in tail tendons as compared to the skin (7.31±0.32 and 5.78±0.45 μg/g, respectively).

It has been proposed in the literature that zinc is likely to share similar binding sites in tissues as lead (Grandjean and Olsen 1982). In our experiments, we observed consistently higher levels of zinc present both in the skin and tendons which contained nearly three times as much zinc as the skin (2.8 times in average).

Massive accumulation of both lead and zinc (with and without zinc administration) was observed in placenta and chorionic membranes. While at low doses of lead (0.1 and 0.2 %) the average was 133 μg per g collagen on the average, animals drinking 0.8% of lead acetate reached the surprising value 1027 μg/g of tissue collagen (calculated as hydroxyproline content x 7.46). Again, the addition of EDTA to drinking water decreased the amount of deposited lead in placenta and chorionic membranes.

The amount of zinc present in placenta and chorionic membranes was about four times higher than the amount of lead in animals administered the low

doses (0.1–0.2 %) of lead acetate. However, in animals administered the 0.8 % dose of the lead acetate, the ratio was reversed and the amount of lead deposited in placenta and chorionic membranes was about 1.5 times the amount of deposited zinc.

The reason for the distinct difference between the amount of lead deposited in the rat skin and tendons as compared to the amount deposited in placenta and chorionic membranes can be discussed from two different aspects. First, the amount of blood perfusing the placenta and chorionic membranes is higher than that reaching the skin, while the lowest blood flow was found in the tail tendon. As the lead reaching the placental stroma must be delivered by the blood, it may be speculated that it is the availability of the metals (i.e. lead and zinc), that causes the difference in the amount deposited compared to other collagen containing tissues. The other point is that the placenta and chorionic membranes differ considerably in their protein composition compared to the other two tissues investigated. This may alter the proportion of available binding sites in proteins and modify the amount of metal deposited.

In order to reveal the proportion of both lead and zinc deposited in the collagenous stroma, the tissues investigated were treated with 6 M urea. The results (Table 3) indicate 68–82 % recovery of lead in the skin and 96–100 % recovery of lead in the tail tendon indicating that there are two unequal pools of bound lead; the smaller pool of the metal is apparently bound to non-collagenous proteins in the skin while virtually no binding of lead to other components than collagen had occurred in the tail tendon. The situation with zinc is similar, yielding very high recoveries both in the skin and tendon. This also applies to the situation when the high dose (0.8 %) of lead acetate was applied. This means that collagen proteins are indeed the main target of lead accumulation in connective tissue. It is noticeable that only in tail tendon samples (both urea treated and untreated) a tendency to zinc depletion was observed with increased administration of lead (non-significant) but no such tendency was observed with the other tissues investigated.

On the other hand, the situation is different in the placenta and chorionic membranes. The recovery in 6 M urea-treated tissues ranged between 47–58 % for lead and 67–81 % for zinc. This means that about 40 % of lead was bound to other than collagenous proteins. On the other hand, recoveries of zinc ranged between 67–81 % indicating that non-collagenous protein-bound zinc is within the range 20–30 % of all zinc present. One point, however, is to be emphasized. In the animals administered 0.8 % lead, the recovery of lead was 58 % and was the highest of all recoveries in the placenta and chorionic membranes. This may be interpreted by assuming that collagen proteins are the target proteins for depositing relatively large amounts of this heavy metal. On the other hand, no increased proportion of collagenous proteins was observed in the tissues investigated (data not shown).

It can be concluded that administration of lead in drinking water to experimental animals leads to the accumulation of this metal in collagen-containing tissues. At low doses, there is practically no difference in the amount of lead deposited. It seems that there is a correlation with the blood supply; well perfused tissues exhibit larger amounts of deposited lead (and also zinc without zinc supplementation). The most pronounced accumulation was shown to occur in the placenta and chorionic membranes. However, a considerable proportion (about 40 %) were bound to non-collagenous constituents of these tissues.

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Reprint requests
Prof. Z. Deyl, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic.