Bioavailability of Biologically Detoxified Lead: Risks Arising from Consumption of Polluted Mussels

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The possible risk for human health arising from consumption of lead-polluted shellfish was suggested by experiments on the bioavailability for mice of a "biologically detoxified" form of the metal. In this work, young mice were fed with a mixed diet including mussels collected in a Pb-polluted area. Metal concentrations in blood, kidney, liver, urine, and feces and the activity of erythrocytic δ-aminolevulinic acid dehydratase were determined after 1, 2, and 4 weeks. Comparisons were made with mice treated with balanced diet, mixed diet including control mussels, and drinking water with lead dissolved as acetate. In mice fed polluted mussels, lead concentrations increased in blood, kidney, liver, whereas no significant accumulation was observed in urine. Different responses in mice treated with Pb(CH3COO)2 in drinking water are probably due to the diversity of lead chemical form in the two treatments. Our results demonstrate the bioavailability of biologically detoxified lead that can be transferred to a consumer with possible consequences also for human health. — Environ Health Perspect 102(Suppl 3):335–338 (1994).

Key words: lead, mussels, mammals, bioavailability, accumulation, excretion

Introduction

Heavy metal pollution along coastal areas is widely monitored by using several marine invertebrates, which concentrate the metals in a detoxified form in their tissues (1–6).

At present, acute metal poisoning from consumption of fish and shellfish is quite unlikely, mainly because marine food is a minor component of diets and shellfish farming is strictly regulated in many countries. However, consumption of mussels collected from polluted environments could represent a risk of chronic intoxication, especially for people living in coastal zones.

In lead-contaminated areas, mussels accumulate large amounts of this metal in the form of insoluble salts, which represent an efficient cellular detoxification mechanism by which the metal becomes unavailable (7–9).

The observation that metals detoxified as insoluble salts in the tissues of marine invertebrates (bivalves, gastropods, and barnacles) are not transferred to carnivorous gastropods (primary consumers) prompted the suggestion that their bioavailability along marine food chains could be re-duced (10).

The uptake and toxic effects of lead in mammals have been extensively investigated by using chemical forms easily accumulated by the organisms. However, the bioavailability of lead ingested by mammals in a biologically detoxified form (such as that of lead-concretions found in mussel tissues) has not been investigated. Information on this particular aspect would allow researchers to assess the risk to human health arising from consumption of contaminated shellfish.

The coastal area of Scarlino (North Tyrrhenian Sea) is highly contaminated by lead, with values of up to 150 µg/g dry weight measured in mussels (11,12). Although shellfish collection is not allowed in this area, the prohibition is usually ignored by the local people. It was thus of interest to compare the accumulation and excretion of lead provided to mice in a biologically detoxified form and as soluble salt.

Materials and Methods

Male CD, Swiss mice (Charles River, Milano, Italy), housed in stainless steel cages, were acclimatized to laboratory conditions for 7 days with tap water and commercial balanced food ad libitum. Mice were then exposed to one of the following treatments for 4 weeks: a.) normal diet (commercial balanced food); b.) mixed diet (including balanced food and commercial mussels); c.) balanced food and lead dissolved as acetate (80 mg/l) in drinking water; d.) mixed diet (including balanced food and polluted mussels). Polluted mussels were collected by scuba divers and control mussels were obtained from a local fish market.

Mussels were boiled and supplied to the animals (about 30 g wet weight per mouse). Lead concentration in mussels was determined by atomic absorption spectrophotometry after nitric digestion according to Regoli and Orlando (12). Mice were sacrificed after 1, 2, and 4 weeks. Feces and urine were collected by keeping the animals in metabolic cages. Following ether anesthesia, blood was collected by incision of the vena cava, and livers and kidneys were removed. All the biologic samples were maintained overnight in 65% nitric acid (Merck Suprapur, Darmstadt, Germany) and boiled in a reflux system for 2 hr. Lead was analyzed by electrothermal atomic absorption spectrophotometry (Varian SpectrAA 300 Zeeman Mulgrave, Australia).

The activity of erythrocytic δ-aminolevulinic acid dehydratase (ALAD) was measured immediately after blood collection, according to the standardized European method (13).

Data were subjected to one-way analysis of variance (ANOVA); the differences between groups of means were then tested with the multiple range test of Scheffe.

Results

The mean lead concentrations measured in control and polluted mussels were, respec-
Table 1. Lead concentrations in blood (PbB), kidney (PbK), liver (PbL), urine (PbU), faeces (PbF) and erythrocytic δ-aminolevulinic acid dehydratase (ALAD) in mice exposed to various treatments (Mean values ± standard deviations, n = 5).

| Treatment of exposure | Weeks | Blood (μg/100 ml) | Kidney (μg/g dw) | Liver (μg/g dw) | Urine (μg/100 ml) | Feces (μg/g dw) | ALAD (μmol/hr RBC) |
|-----------------------|-------|-------------------|------------------|----------------|-------------------|-----------------|-------------------|
| A                     | 0     | 0.80 ± 0.34*      | 2.71 ± 0.64*     | 1.60 ± 0.56*   | 5.12              | 3.56            | 1673 ± 266*       |
| A                     | 1     | 0.74 ± 0.45*      | 3.18 ± 0.31*     | 1.59 ± 0.61*   | 4.38              | 3.70            | 1680 ± 396*       |
| A                     | 2     | 0.92 ± 0.42*      | 2.55 ± 1.17*     | 1.83 ± 0.59*   | 3.70              | 2.02            | 1556 ± 392*       |
| A                     | 4     | 0.66 ± 0.29*      | 2.95 ± 0.83*     | 1.81 ± 0.53*   | 5.40              | 3.26            | 937 ± 465*        |
| B                     | 0     | 0.80 ± 0.34*      | 2.71 ± 0.64*     | 1.60 ± 0.56*   | 5.12              | 3.56            | 1673 ± 266*       |
| B                     | 1     | 0.89 ± 0.63*      | 3.68 ± 1.17*     | 1.75 ± 0.54*   | 4.13              | 5.68            | 1723 ± 200*       |
| B                     | 2     | 0.98 ± 0.34*      | 4.05 ± 1.13*     | 1.82 ± 0.56*   | 6.10              | 6.29            | 1489 ± 222*       |
| B                     | 4     | 0.81 ± 0.38*      | 3.73 ± 1.31*     | 1.77 ± 0.47*   | 5.10              | 4.78            | 769 ± 544*        |
| C                     | 0     | 0.80 ± 0.34*      | 2.71 ± 0.64*     | 1.60 ± 0.56*   | 5.12              | 3.56            | 1673 ± 266*       |
| C                     | 1     | 1.07 ± 2.30*      | 9.12 ± 1.64*     | 2.22 ± 0.75*   | 32.3              | 197             | 1423 ± 258*       |
| C                     | 2     | 1.76 ± 2.41*      | 10.7 ± 3.44*     | 2.11 ± 0.51*   | 33.7              | 126             | 1138 ± 345*       |
| C                     | 4     | 18.1 ± 1.44*      | 11.8 ± 5.31*     | 2.34 ± 0.56*   | 51.9              | 271             | 703 ± 328*        |
| D                     | 0     | 0.80 ± 0.34*      | 2.71 ± 0.64*     | 1.60 ± 0.56*   | 5.12              | 3.56            | 1673 ± 266*       |
| D                     | 1     | 11.8 ± 2.30*      | 11.3 ± 3.44*     | 5.11 ± 1.87*   | 5.70              | 40.0            | 1421 ± 258*       |
| D                     | 2     | 15.4 ± 4.95*      | 17.4 ± 1.55*     | 7.96 ± 1.59*   | 4.90              | 34.4            | 740 ± 226*        |
| D                     | 4     | 19.4 ± 5.92*      | 22.3 ± 4.98*     | 12.0 ± 3.62*   | 6.10              | 137             | 699 ± 231*        |

* values obtained from analysis of 1 sample constituted by urine or feces of 5 animals. A: normal diet; B: mixed diet with control mussels; C: lead dissolved as acetate in drinking water; D: mixed diet with polluted mussels. *, §, †: values marked in each treatment by a different symbol are significantly different from each other at the 5% level of significance.

Discussion

The total daily intake of lead from food may vary widely among human populations and individuals according to different
dietary habits although the World Health Organization, in 1980, determined (14) that the maximum weekly intake of lead tolerated by an adult man was 3 mg. Polluted mussels showed a mean Pb concentration of about 60 μg/g dry weight (10 μg/g wet weight). Consequently, consumption of 300 g of these marine animals would correspond to the maximum permissible weekly dose of lead. For this reason it is important to know the real Pb bioavailability ingested by a mammal in a biologically detoxified form.

The lead level in blood is considered one of the best indicators of current exposure (14,15) and it is usually dose-related to several toxic effects. In children, for example, neurological and neurobehavioral diseases have been reported for blood lead concentrations ranging from 20 to 50 μg/100 ml (16-20).

In addition, many studies have demonstrated that in adults PbB below 30 μg/100 ml can affect blood pressure with possible effects on cardiovascular health (21). A more detailed description of the relationships between lead blood levels and erythropoietic, neural, renal, endocrine and hepatic effects has been given by EPA (19).

In this work, mice fed with polluted mussels showed a net increase of blood lead concentration with values (up to 20 μg/100 ml after 4 weeks) very similar to those measured in mice treated with lead acetate in drinking water. Higher levels of PbB, recently obtained by Flora and Tandon (22), Piasek et al. (23), Tomokuni et al. (24) who reported respectively values of 78, 74, and 47 μg/100 ml after 8, 14, and 2 weeks exposure, are probably related to longer exposure times or higher lead doses.

Although in our experiments the daily intake of lead in mice fed with polluted mussels cannot be precisely quantified because consumption was largely variable during the experiment, a maximum daily digestion of about 10 g per mouse (corresponding to 100 μg Pb) has been estimated. On the other hand, a maximum Pb daily intake of 640 μg has been evaluated for mice treated with lead dissolved in drinking water. Although the increase of PbB cannot be related to a well-defined dose of ingested lead, our results clearly demonstrate that lead accumulated and detoxified by mussels can become bioavailable in mice and consequently represents a possible risk for mammals who consume these polluted molluscs.

Lead has several biochemical effects that interfere with many enzymes involved in the heme synthesis such as the erythrocytic δ-aminolevulinic acid dehydratase which, in this respect, is considered one of the best indicators of recent lead intoxication (25-27). This enzyme is totally inhibited at blood lead concentrations higher than 70 to 80 μg/100 ml (28), although a PbB concentration of 10 μg/100 ml reduces ALAD activity in 10% of persons (29).

In recent studies, mice treated with lead dissolved in drinking water (500 mg/l) or injected intraperitoneally (200 μmole/kg) exhibited a reduction of ALAD activity of 90% (24) and 95% (30). In the present work, ALAD activity decreased by 15% after 1 week, in both the treatments (contaminated drinking water and polluted mussels), whereas the enzyme activity, after 2 weeks, was reduced by 55 and 30% respectively, in mice fed with polluted mussels and in those treated with lead in drinking water. These results indicate a toxic effect induced by consumption of polluted mussels. More difficult to explain is the reduction of ALAD activity in all the groups of mice after 4 weeks, probably related to some effects of the captivity period in the laboratory on heme biosynthesis. Lead concentrations in the kidneys increased in both groups of mice exposed to the metal. This evidence agrees with the findings that kidney, where lead deposition is usually not greater than 3%, becomes one of the main target organs in the case of large amounts of lead ingested over a short period (31).

In this study a significant excretion of lead through the urine is showed only by mice treated with lead in drinking water (PbU over 500 μg/100 ml), whereas mice supplied with polluted mussels show no significant difference from the controls. Lead in urine is usually dependent on the level of Pb in blood (32) and, in this respect, the low concentration in urine of mice fed with polluted mussels may be due to a different metabolic pathway of the metal related to its chemical form. However, concentration of lead in urine is affected by many unknown factors, so it does not allow any conclusion about previous exposure and absorption of the metal (15).

Duran et al. (33) showed that a large proportion of lead that penetrates into the organism reaches the liver; there it is partially metabolized and partially excreted with bile into the intestine, where only a
small percentage is reabsorbed (34). In this study a net increase of lead concentration in liver was observed only in mice fed with polluted mussels. The diversity of lead behavior in mice exposed to the two treatments is probably due to the different chemical form which, in mice supplied with polluted mussels, reduced the urinary excretion of lead but increased its accumulation in the hepatocytes.

In conclusion, the accumulation of lead in mice fed with polluted mussels clearly indicates the high bioavailability of the metal in its biologically detoxified form and a possible risk to human health arising from consumption of molluscs collected in polluted environments.

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