Mercury content in rat teeth after administration of organic and inorganic mercury

The effects of interrupted exposure and of selenite

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Rat molars are indicators of exposure concentration and target organ content in chronic mercury vapor exposure. We wished to study the accumulation and persistence of organic and inorganic mercury in rat teeth and the effect of selenium on mercury retention. Male Wistar rats received either inorganic or organic mercury (with or without addition of selenite), selenite only, or no mercury or selenite (controls) in the drinking water for 4 weeks. Group A was killed after exposure. Group B was killed 20 weeks later. The mercury content was measured by cold-vapor atomic absorption spectrophotometry. The mercury content in the molars in group B was 66% and 77% less than in group A after inorganic and organic exposure, respectively. In the incisors the corresponding reductions were 90% and 97%. Selenite had limited effect on mercury retention in group A and none in group B. We suggest that rat molars and, by inference, human deciduous teeth may serve as indicators of organic and inorganic mercury exposure. □ *Environment; tooth; toxicology; trace elements*

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Mercury occurs widely in nature and has long been known as a toxic element. The usual indicators for the mercury content of target organs in mercury exposure are urine, blood, and hair. Blood and urine are most reliable in recent exposures and tell us little about the long-term exposures (1). Hair is a well-established indicator of organic mercury exposure but is less reliable for inorganic mercury (1, 2). Its usefulness as an indicator is also limited by the large variability in hair length between individuals. Hair grows at a rate of approximately 1 cm per month, and material covering large time spans would thus not be available for investigation from large parts of the population (including many children).

An indicator suitable for large epidemiologic studies of the influence of environmental mercury would be useful. Dental hard tissues have a very low biologic turnover. Human deciduous teeth are more suitable than permanent ones as indicators since they represent a shorter and more easily verifiable exposure period. They are also discarded at approximately the same time in all humans, and the collection of such teeth presents no ethical problems. Deciduous teeth are easily available for large studies of both present and past populations (3–5).

Dental hard tissues have been shown to function as indicators of several metals, among them lead and cadmium (3, 4, 6). Mercury has been found by cold vapor atomic absorption spectrophotometry (CVAAS) in unfilled human deciduous teeth (7). Mercury has also been detected by CVAAS in molars from rats exposed to graded doses of mercury vapor (8). A correlation between mercury levels in the molars and the exposure concentration suggested that rat molars can act as indicators of chronic mercury vapor exposure. Correlations were also found between the molars and the target organs kidney cortex and cerebrum.

It is conceivable that organic and inorganic mercury salts are also incorporated into dental tissues. To our knowledge the mercury content of rat teeth after such exposure has not been investigated so far. It would be of value to quantify such an uptake and to investigate the extent to which mercury persists in the dental tissues after the exposures have ended. This would indicate the potential of rat teeth and, as a corollary, also of human primary teeth as indicators of interrupted mercury exposure.

Selenium (Se) is an essential trace element in normal metabolism and is an integral part of the enzyme glutathione peroxidase (9). It has been reported that Se can protect against intoxication from several metallic compounds, including organic and inorganic mercury (9, 10). On selenium addition the tolerance of experimental animals towards mercury has been shown to

Table 1. Median mercury content with maxima (Max) and minima (Min) in rat molars after administration of inorganic or organic mercury combined with selenite

Group	Period	Median	Max	Min
1 ($HgCl_2$)				
A	4 weeks	0.456	0.560	0.403
B	24 weeks	0.157	0.207	0.042
2 (CH_3HgCl)				
A	4 weeks	0.888	1.850	0.700
B	24 weeks	0.201	0.210	0.081
3 ($HgCl_2 + Na_2SeO_3$)				
A	4 weeks	0.473	0.681	0.364
B	24 weeks	0.179	0.314	0.111
4 ($CH_3HgCl + Na_2SeO_3$)				
A	4 weeks	1.438	2.662	1.218
B	24 weeks	0.133	0.321	0.085
5 (Na_2SeO_3)				
A	4 weeks	0.010	0.077	0.008
B	24 weeks	0.013	0.016	0.010
6 (H_2O)				
A	4 weeks	0.021	0.151	0.006
B	24 weeks	0.011	0.020	0.008

* All values are in μg Hg/g tooth substance. The animals were exposed for 4 weeks. Group A was killed 24 h after the end of the exposure, whereas group B survived for 20 weeks before being killed.

Table 2. Median mercury content with maxima (Max) and minima (Min) in rat incisors after administration of inorganic or organic mercury combined with selenite

Group	Period	Median	Max	Min
1 ($HgCl_2$)				
A	4 weeks	0.050	0.135	0.029
B	24 weeks	0.004	0.005	0.003
2 (CH_3HgCl)				
A	4 weeks	1.168	1.978	0.864
B	24 weeks	0.032	0.053	0.024
3 ($HgCl_2 + Na_2SeO_3$)				
A	4 weeks	0.093	0.159	0.064
B	24 weeks	0.003	0.003	0.002
4 ($CH_3HgCl + Na_2SeO_3$)				
A	4 weeks	1.290	1.620	1.045
B	24 weeks	0.025	0.048	0.024
5 (Na_2SeO_3)				
A	4 weeks	0.008	0.011	0.005
B	24 weeks	0.003	0.035	0.003
6 (H_2O)				
A	4 weeks	0.005	0.020	0.003
B	24 weeks	0.004	0.005	0.003

* All values are in μg Hg/g tooth substance. See also legend to Table 1.

increase, with a concomitant increase in the retention of both elements (11). It would be of interest to establish whether an increased selenium supply leads to increased mercury retention in teeth.

The aim of this study was therefore to quantify the incorporation of organic and inorganic mercury in rat teeth and to assess the extent to which the mercury persisted after the end of the exposure. It was also our intention to study the effect of selenium on mercury retention.

Materials and methods

Two groups (A and B), each with 42 male Wistar rats (Møllegaards Breeding Centre Ltd., Ejby, Denmark), were used in this study. The rats were 3 months old at the start of the experiments and had an initial average body weight of 180 g. They were fed Altromin 1324 (Altromin Specialfutterwerke, Lange, Germany). The animals in each group were randomly divided into six subgroups (A1–A6, B1–B6) consisting of seven animals each (see Tables 1 and 2). One subgroup of each main group received 20 mg $HgCl_2$/l in the drinking water every 2nd day, whereas another received 20 mg CH_3HgCl/l in the same manner. Two subgroups were treated in the same manner, with the additional administration of 2 mg Na_2SeO_3/l in the drinking water on the days when they were not receiving mercury. Mercury and selenite were given on alternate days to avoid precipitation of HgSe complexes, which would result in reduced absorption. One subgroup received 2 mg Na_2SeO_3/l in the drinking water every 2nd day, and the last subgroup received tap water every day and served as control.

The animals in group A were exposed for 4 weeks and were killed 24 h after the end of the exposure. The rats in group B were exposed for 4 weeks, followed by 20 weeks of non-exposure before being killed. At the time of death, the animals were anesthetized with sodium pentobarbital (50 mg/kg body weight intraperitoneally) before transcardial perfusion at 120 mmHg for 12 min with a fixative containing 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at room temperature.

The jaws were removed, and the incisors and molars were extracted. The teeth were then treated in accordance with a method previously described (7). In brief, the teeth of the left side of the jaw were washed in 0.01 N HCl, rinsed in distilled water, and allowed to dry before digestion. They were then pooled in two groups, one for incisors and one for molars for each animal, dissolved in 2 ml 2 N HCl, and stored for 6 days at 50 °C in corked and sealed polypropylene test tubes.

The samples were analyzed by CVAAS with a Perkin-Elmer 370A atomic absorption spectrophotometer equipped with an MHS 20 mercury hydride system. The results were recorded graphically. Sodium borohydride was used as the reducing agent (12, 13). Ten samples of IAEA H-5 Animal Bone were also analyzed. The mean and standard deviations were 0.009 and 0.003 μg/g Hg, respectively. The CV was 36.2%. The IAEA has not found a certified value for mercury in animal bone but has provisionally accepted values between 0.002 and 0.014 μg/g for the H-5 material. The coefficient of

variation (CV) of the mercury values found for 10 samples of homogenized tooth powder from human premolars was 6.6%.

The SPSS/PC+ V.3.0 (SPSS, Inc., USA) statistical computer package was used for statistical analysis of the results. As each exposure subgroup was small, the Mann–Whitney test was used to test for differences between pairs of subgroups, and the Kruskal–Wallis one-way ANOVA test was used when testing more than two subgroups (14).

Results

The teeth from the subgroups that had received mercury in group A had higher levels of mercury than the corresponding subgroups in group B (Tables 1 and 2). Comparison between the groups exposed to mercury for only 4 weeks and the groups exposed for 4 weeks followed by an unexposed period of 20 weeks showed a reduction in the mercury content of the molars by 66% for the inorganic and 77% for the organic group. In the incisors the corresponding reduction was 90% and 97%. The difference between groups A and B was always statistically significant (Tables 1 and 2). The difference was not significant for subgroups 5 and 6 (Tables 1 and 2).

All subgroups that had received only mercury had significantly higher levels of mercury than the controls (subgroup 6) except the incisors in the rats exposed to inorganic mercury in group B. The Kruskal–Wallis test showed that the variation between the subgroups that had also received selenite (subgroup 3 or 4) and between the selenite only (subgroup 5) and the control group (subgroup 6) was significant in all cases except the incisors in group B.

The addition of selenite to inorganic mercury resulted in no significant increase in the uptake of mercury in the molars of group A. The corresponding incisors showed a slight but significant increase in the two groups. The differences for the subgroups receiving organic mercury were small: significant for the molars but not for the incisors.

Neither molars nor incisors showed any significant differences between the mercury and the mercury/selenite subgroups 20 weeks after the end of the exposure (group B).

Discussion

The present study has shown that mercury is accumulated in rat teeth after administration of either organic or inorganic mercury. This is important, since the largest part of mercury in marine food is organic mercury (1, 2). Inorganic mercury is the most important type of mercury in non-marine food and in drinking water and can also be important in industrial environments (1, 2).

The large decrease in mercury content in the rat incisors 20 weeks after the end of exposure is probably due to the rapid, constant eruption of the teeth. These are renewed completely within 40–50 days (15). The rat incisors thus do not reflect past mercury exposure.

Selenite has been shown to reduce the toxicity of both organic and inorganic mercury in rats (1, 16). The effects might be due to a redistribution of mercury (1, 17) or to a change in the way mercury is bound to proteins (17). An increase in the mercury content in organs such as blood, liver, and testis was seen in rats after combined exposure to mercury and selenite, whereas other organs had a decrease in mercury content (18). The total amount of mercury retained was not altered. Increased retention of methyl mercury has also been seen in adult and fetal brains after selenium administration (1).

Increased concentrations of both selenium and mercury have been reported in thyroid, pituitary glands, kidneys, and brain tissues in human autopsy cases when there is a history of mercury exposure (19).

Selenite interaction with inorganic and organic mercury differs (9). The slight but statistically significant differences in mercury content found for the organic mercury in molars and the inorganic mercury in incisors after selenite administration in group A may be due to possible differences in selenite retention in incisors and in molars. Since no statistically significant difference was found for the animals in group B, any effect of selenite on the use of rat teeth as indicators must be small.

The lower mercury content in the molars of group B than in group A may have several explanations: much of the mercury in pulp tissue will have been excreted, with the possible exception of the mercury in the odontoblasts (20), but we do not know the extent of mercury deposition in the cirumpulpal dentin. In addition, a dilution effect exists in rat molars due to the changes taking place in the teeth after the end of the exposure. Some of the tooth mass containing mercury is lost because of attrition. Studies have shown that 6- to 12-month-old rats can have their anatomic crown heights reduced by as much as 25–50% (21, 22). Simultaneously, deposition of secondary dentin and apical cementum increases the mass of calcified tissue in the tooth (21–23). The secondary cementum makes up about one-third or more of the roots in the adult rat (21, 23). Consequently, tooth substance with a potentially high content of mercury is lost while new tissue with lower mercury content is formed. These changes are much greater in rat molars than in human teeth (24).

Wistar rats have an average life expectancy of 27–30 months (25). The exposures ended when the animals were approximately 4 months old, and the animals of group B were killed at around 9 months. This means that after a period corresponding to one-fourth to one-fifth of the animal's life span, 34% of the inorganic and

23% of the organic mercury were still present in the molars. Calculation of halftimes ($T_{\frac{1}{2}}$) based on a simple one-compartmental model gives values of 91 days for inorganic mercury and 65 days for organic mercury. On the other hand, if halftimes based on a linear model caused by attrition are calculated, values of 106 days and 70 days are found. Thus, the two models give similar results for rat molars.

Inorganic mercury has large variations in elimination rates between organs. Brain, kidneys, and testicles have the longest retention times (1). Halftimes varying from a few hours to 141 days have been reported, depending on organ and compartmental model (1, 26–31). The distribution of metallic mercury after oxidation approximates that of inorganic mercury and will in many ways have a similar elimination pattern (1).

Organic mercury has a more even distribution among the organs in humans than does inorganic mercury and has small variations in excretion rates between the organs (32). About 1% of the organic mercury body-burden in humans is excreted each day. This corresponds to a biologic halftime of 70 days (1). Variations between 6 and about 70 days, using one- or two-compartmental models, are reported (31, 33, 34).

The halftimes of both the organic and inorganic mercury in rat molars are similar to those reported in the literature for main target organs. Although the mechanism of mercury reduction may be different, the mercury content of these teeth is reduced as if they belonged to compartments of mercury with long halftimes. It is therefore possible that rat molars could act as indicators for the mercury content in organs with long retention times.

Mercury has previously also been detected in rat molars by CVAAS in continuous mercury vapor exposure (8). The study showed that the levels were correlated with the exposure concentration and with the concentration in several target organs. It was suggested that rat molars can act as indicators for long-term, chronic mercury vapor exposure. Correlations between the mercury content of rat molars and target organs after interrupted exposure were not directly shown in this study. However, it should be mentioned that in another study we have found a clear correlation between the mercury content in kidney cortex and molars in young rats after interrupted exposure ($r = 0.78$, $p < 0.001$) (35).

Rat molars are used as models in this study as they are comparable to human deciduous teeth with regard to time of mineralization and eruption (15, 23). On the basis of our results, we suggest that mercury absorbed in organic and inorganic form may accumulate in human deciduous teeth. Furthermore, as the animal studies also suggest that rat molars may act as indicators for the mercury exposure of the animals, such a situation might also exist for human deciduous teeth. Since both attrition and deposition of secondary dentin and cementum take place at a much slower rate in humans than in rats (23, 24), one might expect that the loss of mercury in human deciduous teeth after cessation of exposure would be smaller than in the rat molars and consequently give a better indication of the original exposure.

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