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Dental amalgam mercury exposure in rats

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Abstract

The aim of this study was to measure the distribution of mercury, in tissues of rats exposed to amalgam over a two months period. Possible interaction of mercury with copper and zinc in organs was also evaluated. Rats were either exposed to mercury from 4 dental amalgams, or fed the diet containing powdered amalgam during two months. Mercury was measured in the kidney, liver and brain, copper in kidney and brain and zinc in kidney. The results showed significantly higher concentrations of mercury in the kidneys and the brains of rats in both exposed groups compared to control. Even after two months of exposure to mercury brain mercury concentration in rats with amalgam fillings was 8 times higher than in the control and 2 times higher than in rats exposed to amalgam supplemented diet. The highest mercury concentration in the latter group was found in the kidneys and it was 5 times higher than in the control group. We found no significant differences between mercury levels in exposed and control rat's liver. Exposure to mercury from dental amalgams did not alter the concentrations of copper and zinc in the tissues. Histopathological analyses of rats tissues did not show any pathological changes. These results support previously proposed nose-brain transport of mercury released from dental amalgam fillings.

Introduction

Many studies on humans (Hanson & Pleva 1991, Halbach 1995, Evans 1998) and animals (Danscher *et al.* 1990; Hahn *et al.* 1990) demonstrated that dental amalgam fillings continuously emit mercury vapour. Therefore, dental amalgam is a major source of exposure to mercury for general population (Weiner 1990; WHO 1991). Mercury from dental amalgam fillings may be absorbed into the body through the lung (Patterson *et al.* 1985), mucous membrane (Hahn *et al.* 1989) or the digestive tract (Eley & Cox 1993). Recently, the direct transport of mercury from the oronasal cavity to the brain was proposed (Störtebecker 1989a; Störtebecker 1989b). Mercury vapour rapidly crosses cell membranes and after oxidation to ionic mercury, it accumulates in various tissues. In primates, exposed to mercury through dental amalgam fillings depositions of mercury were found in the endocrine glands (pituitary, thyroid, adrenal), brain, liver, kidneys and lungs after short or long term exposure (Hahn *et al.* 1990; Danscher *et al.* 1990). In subjects occupationally exposed to mercury vapour (dentists and miners) the highest concentrations of mercury were found in *post mortem* studies in the pituitary and thyroid glands, brain and kidneys (Kosta *et al.* 1975; Nylander & Weiner 1991). Human autopsy studies demonstrate significantly higher mercury level in the brain and kidney of humans with aged amalgam fillings than in subjects with no amalgams (Nylander *et al.* 1987). The effect of inorganic mercury on the copper and zinc level in the rats kidney in chronic mercury intoxication has been reported previously (Bogden *et al.* 1980; Škreblin *et al.* 1988; Muto *et al.* 1991; Blanuša *et al.* 1994). However, there is no information available on the mercury-essential trace elements interaction in an-

imals exposed to mercury through dental amalgam fillings.

The purpose of this study was to evaluate the distribution of mercury in rats after two months exposure to mercury from amalgam fillings or given through the diet and to look for any evidence of renal, liver or brain pathology. Possible interaction of mercury with zinc and copper was also evaluated.

Materials and methods

Animals

The experiments were performed on 30 male, Wistar rats obtained from the Pliva Pharmaceutical Co (Zagreb, Croatia) breeding farm. During the experiments the highest principles of animals welfare were obeyed and approved by ethic committee. At the beginning of the experiments, rats were 3 months old with an average body weight of 300 g. Rats were divided into three groups according to the treatment: control and two experimental groups. Control (C) rats received the standard diet (Pliva, Zagreb, Croatia) and were kept in the same laboratory with the exposed animals. The animal room was equipped with automatic light cycles (12 hours light/dark). Relative humidity and temperature were maintained within a range of 50 to 70% and 22 to 25 °C, respectively. During the experiments rats were kept in polycarbonate cages (2 animals per cage). Standard rats diet and drinking water was provided *ad libitum*. Body weights were recorded daily.

We used conventional type non-gamma 2 amalgam containing preamalgam powder (Ag 70.1%, Sn 18%, Cu 11.9%, by weight, Amalcap Plus, Vivadent, Schaan, Liechtenstein) and mercury in ratio of 1:0.97. This amalgam was implanted either into the teeth (Group 1) or ground and mixed with the regular diet (Group 2).

Group 1: Each rat was implanted 4 occlusal dental amalgam fillings, placed in front teeth under Chloral hydrant anaesthesia using a standard dental procedure. The average mass of amalgam filling in each tooth was 75 mg containing 50% of pure elemental mercury. The total amalgam mass was 300 mg in each animal. All fillings were worn out by grinding during the 2 months experimental period.

Group 2: Rats were fed regular (powdered) diet mixed with powdered amalgam (8 mg/day). The amalgam was triturated and hashed up, and then mixed with the standard laboratory diet. The daily dose of powdered amalgam in the diet was matched to similar quantity of dental amalgam in Group 1. All rats were sacrificed under urethane anaesthesia on day 60 after the beginning of the experiment. Liver, kidneys and brain were dissected for mercury, copper and zinc analysis.

Determination of mercury, copper and zinc

Mercury concentration was determined by a modified method described by Farant *et al.* (1981). Samples (1 g) of the right kidney and brain were wet digested with 2 ml of concentrated nitric acid at 80 °C in closed ampules. Samples were heated for 5 hours in a programmed digestion system (DS-40, Tecator, Sweden). Mercury was analysed using the cold-vapour atomic absorption spectrometry method (CVAAS) on Mercury Monitor LDC (Milton Roy, Riviera Beach, FL). Mercury analysis in each sample was repeated 2–4 times. The detection limit of the method was 0.4 ng/ml for analyte solution, i.e. 4 ng/g wet weight of tissue. The precision of the method, calculated as the coefficient of variation within- and between day was 9.2 and 12%, respectively. The accuracy of the method was established by analysing the standard reference materials: horse kidney H-8 (IAEA, Austria) and pig kidney CRM-186 (BCR, Brussels, Belgium). Recovery obtained was 103% and 98% of the declared reference values, respectively.

Copper in the kidneys and brain and zinc in kidneys were determined by flame atomic absorption spectrometry in the same solutions after mercury analysis. Varian AA 375 instrument with deuterium background correction was used for this purpose. Detection limits for Cu and Zn were 0.24 and 0.18 μg/g, respectively. The method was verified on certified standard reference material of SRM bovine liver 1577b (NIST,USA). The recovery obtained for copper was 111 and for zinc 109% of the reference value.

Histopathological examination

Tissues (kidney and parts of liver and brain) were collected from control and both exposed groups of rats. Samples were routinely fixed in 10% buffered formalin according the procedure described previously (Paget & Thomson 1979). Sections 4 μm thick were stained with haematoxylin and eosin and examined in light microscopy. In some cases the slides were stained with Mallory trichrome and Giemsa.

Statistical evaluation

The results were expressed as arithmetic means±SEM. The differences between the groups were tested by the analysis of variance followed by Duncan's multiple range test (CSS-Statistica release 3.1 Statsoft 1991-ANOVA/MANOVA). The differences at the $p < 0.05$ level were taken as significant.

Results

Body weights of animals did not differ significantly between any of groups at the end of the experiment. The consumption of food and water did not differ between groups either.

Table 1 shows the concentrations of mercury in the kidney, liver and brain of rats exposed to mercury from amalgam fillings (Group 1). The results show that 2 months after the placement of dental amalgams the concentrations of mercury in the brain and kidney were 0.62 and 0.56 μg/g, respectively (in the controls respective concentrations were 0.08 and 0.24 μg/g, respectively), that is, 8 and 2 times higher than control values. The data were compared with those obtained for rats exposed to amalgam through diet (Group 2). The latter group, after the same time of exposure, mercury concentrations were 0.25 μg/g in the brain and 1.09 μg/g in the kidney (Table 1). Those values were also significantly higher than values obtained for control group (3 and 5 times, respectively). Our results show that the highest concentration of mercury was found in the brain of rats with amalgam fillings and in the kidneys of rats exposed to amalgam through diet. The differences in mercury kidney and brain concentrations between two experimental groups were also significant.

Figure 1 shows the concentrations of copper and zinc in the kidneys and brain together with respective control values. The zinc concentration in the kidney did not differ significantly between the control and the exposed groups. Although slightly higher than in control group, kidney copper concentration in dietary exposed rats did not differ significantly from other groups. Histopathological findings in all organs were normal.

Table 1. Concentrations of mercury (μg/g wet mass) in organs of rats exposed to mercury from dental amalgam fillings (Group 1) or from diet (Group 2) during 2 months

	Control	Group 1	Group 2
Kidney	0.24 ± 0.16^{a}	0.56 ± 0.07^{b}	1.09 ± 0.12^{c}
Brain	0.08 ± 0.01^{a}	0.62 ± 0.06^{c}	0.25 ± 0.03^{b}
Liver	0.11 ± 0.04^{a}	0.11 ± 0.01^{a}	0.12 ± 0.02^{a}

Results are presented as arithmetic means ± SEM of 10 animals in each group. In Group 1, rats were exposed to mercury from 4 dental amalgam fillings. In Group 2, rats were fed the diet supplemented with 8 mg of Hg daily in the form of amalgam. Control animals received standard diet.
^{a,b,c}Significant differences between groups (at $p < 0.05$ by Duncan's multiple range test) are indicated by different superscript letters.

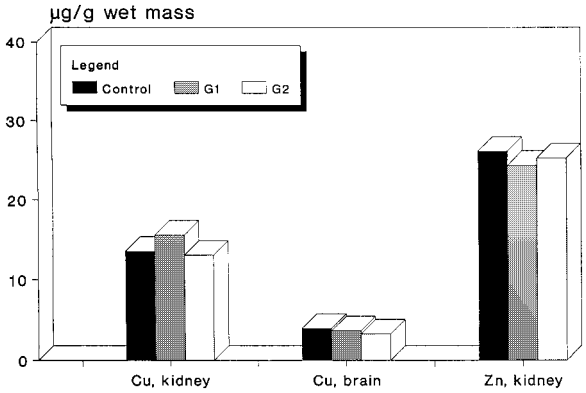

Figure 1. Copper and zinc in rats kidney and brain exposed to mercury from dental amalgam fillings in Group 1 (G 1) or through diet contained powdered amalgam in Group 2 (G 2) during two months.

Discussion

Previous studies performed on monkeys, sheep and rats exposed to mercury through dental amalgam fillings showed that the highest level of mercury was found in the kidneys (Hahn *et al.* 1989, 1990; Danscher *et al.* 1990; Arvidson *et al.* 1994). However, the brain and pituitary gland also showed evidence of mercury accumulation after short or long term exposure to dental amalgam (Hahn *et al.* 1989, 1990; Arvidson *et al.* 1994). In *post mortem* studies performed on dentists, concentrations of mercury in the pituitary and thyroid gland, occipital and renal cortex were higher than in general populations. The differences were most prominent in pituitary glands (Nylander 1986; Nylander & Weiner 1991). Studies on animals indicate that inorganic mercury penetrates the blood-brain barrier only to a very limited extent (Friberg 1989). Therefore, the reason for high accumulation of mercury in the brain of animals and humans could be due to the additional uptake of mercury by a direct nose-brain transport (Störtebecker 1989ab). Such direct transport from the oro-nasal cavity to the brain

was found for other metals such as aluminium (Perl & Good 1987) and cadmium (Tjälve *et al.* 1987). In a recent study Maas *et al.* (1996) investigated the transport of mercury from oro-nasal to the cranial cavity in 55 deceased persons with amalgam fillings. The highest mercury concentrations were detected in the kidney, followed by the pituitary gland, olfactory bulb and trigeminal ganglion. Results of their study do not support the hypothesis of a significant flow of mercury from dental amalgam fillings to the cranial cavity and regression analysis did not reveal significant correlation between the number of dental amalgam fillings and the mercury concentration in these tissues. However, a significant correlation between the number of dental amalgam fillings and the mercury concentration in the kidney was found (Mass *et al.* 1996). Our results show that mercury from dental amalgam fillings accumulates in the brain at a higher rate than after ingestion through diet. Even two months following amalgam placement in rats teeth, the concentration of mercury in the brain was 8 times higher than in control, and 2.5 times higher than in rats exposed to diet supplemented with powdered amalgam.

The analysis of mercury in tissues of rats fed the diet supplemented with powdered amalgam (Group 2) showed that mercury was distributed in a pattern typical for ingested, inorganic mercury (WHO 1991) and the highest accumulation was found in the kidney (Table 1). These results indicate that the distribution of mercury depends on the route and specific chemical form of mercury exposure. Liver mercury concentrations did not differ between the groups.

One of the many physiological effects of mercury is to alter the metabolism of some essential metals. The effect of inhaled mercury vapour and ingested mercury salts on the accumulation of copper in rats kidneys has been reported previously (Bogden *et al.* 1980; Škreblin *et al.* 1988; Blanuša *et al.* 1994). This was explained by association of mercury with metallothionein (MT) in some organs. Higher production of this protein is the consequence of its protective role to heavy metal toxicity (Cherian & Nordberg 1983; Webb & Cain 1982). Rat renal MT contains copper and zinc and association of mercury with MT in kidneys of rats results in higher concentration of copper (Elsenhans *et al.* 1991). In all this reports the level of exposure to mercury and the concentration of mercury in the kidneys was much higher than in our present experiment. On the other hand, Eley and Cox (1986) showed the release of small quantity of copper together with mercury following subcutaneous implantation of high copper dental amalgam to ginea pigs. In our study the rats were also exposed to copper released from high copper level amalgams (11.1%). Therefore, one of the important aspects of this study was to analyse copper in rats organs. However, the influence of copper either released from high copper dental amalgam (Group 1) or ingested through diet supplemented with amalgam (Group 2) and possible mercury-copper interaction on copper level was not found. Results show that concentration of copper in tissues of both exposed groups of rats were not significantly higher than in control group. There was also no significant interaction found with zinc in the kidney of exposed rats. The results of histopathological analysis confirmed no morphological changes in any of tissue due to low level of mercury exposure.

In conclusion, exposure to inorganic mercury through amalgam fillings and diet results in different distribution of mercury in organs. We can also conclude that mercury exposure from amalgam fillings in our experimental conditions does not induce alternations in copper and zinc levels. Further research should focus on the effect of dental amalgam on the level of trace elements with emphasis on chronic, low-level exposure.

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