Blood mercury following DMPS administration to subjects with and without dental amalgam

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Abstract

The use of DMPS as a diagnostic tool in patients with symptoms allegedly caused by mercury from dental amalgam fillings is disputed. We have previously shown that the mercury concentrations in urine cannot be used in such a way. In the present study, we wished to evaluate the effect on blood mercury levels (B-Hg) following intravenously injected DMPS in four groups of subjects: 19 controls without amalgam experience; 21 healthy controls with amalgam fillings; 20 patients with self-reported symptoms from existing dental amalgams; and 20 patients who had removed amalgam fillings. A single dose of DMPS (2 mg/kg) was injected. Blood samples were collected prior to the injection and after 15, 30, 120 min, and after 24 h, and mercury was analyzed by cold vapor atomic absorption spectrophotometry. All groups showed an initial drop of 24 to 30% in the blood levels, approaching baseline values (2.5–5.5 μg/l) after 2 h. The subjects with no amalgam experience had the lowest mercury values. There was no significant difference between the three groups with such experience. There were no significant differences between the two groups with amalgam fillings present. Patients with symptoms allegedly caused by amalgam were not different from the control groups. There were indications that part of the urinary mercury excreted during the first 30 min originated from blood.

Keywords: DMPS; Blood; Dental amalgam; Mercury; Adverse effects

1. Introduction

Amalgam fillings release mercury in vivo (Vimy and Lorscheider, 1985; Olsson and Bergman, 1992). It is generally assumed that the major absorption route is inhalation of evaporated mercury. Approximately 10% of the mercury vapor released from the fillings is transported to the lungs where 80% is absorbed (Olsson and Bergman, 1992; Berglund and Molin, 1996; Mackert and Berglund, 1997). Less than 5% of the mercury released from the fillings is absorbed in the gastrointestinal tract (Mackert and Berglund, 1997; af Geijerstam et al., 2001). In a number of studies, correlations between mercury concentrations in human organs, blood, and urine and the presence of amalgam fillings (Nylander et al., 1987; Langworth et al., 1988; Ahlqwist et al., 1995), have been shown on a group basis.
The media used for biological monitoring of mercury exposure are mainly blood and urine. The concentration of inorganic mercury in blood, mainly resulting from the presence of amalgam fillings, is in the range of 1.3–4.3 μg/l, and in urine 1.4–4.8 μg/l (Weiner and Nylander, 1995; Oskarsson et al., 1996; Sandborgh-Englund et al., 1998; Vannes et al., 2000). It has been maintained that mercury at these levels can cause adverse biological effects (Siblerud and Kienholz, 1994; Richardson and Allan, 1996; Lorscheider and Vimy, 1993; Leong et al., 2001). Some patients reporting oral and/or general symptoms such as headache, joint pain, vertigo, memory problems, fatigue, and sleep disturbances, relate their symptoms to amalgam restorations (Hanson and Pleva, 1991). Although a relation between oral lichenoid reactions and amalgam fillings has been shown to exist, no conclusive evidence has been presented to substantiate the claim that mercury from dental amalgam constitutes a general health hazard (Ahlqwist et al., 1993; Department of Health and Human Services, 1993). However, the concentrations of mercury in blood and urine appears to be of little diagnostic value in differentiating between subjects with or without complaints self-related to amalgam restorations (Berglund and Molin, 1996; Bratel et al., 1997). On the other hand, a Health Canada risk assessment recommends a maximum of four amalgam fillings in adults and less in children (Richardson and Allan, 1996).

DMPS (2,3 dimercaptopropane-1-sulfonate), is a water soluble chelating agent that was first synthesized in the USSR (Petrunkin, 1956). Chelation is the formation of a complex containing at least one polydentate ligand forming a ring of atoms that include the metal atom. The metals are mainly excreted with the drug through the kidneys. The DMPS is given intravenously, or more commonly per os. Approved indications for the use of DMPS are mainly treatment of lead and mercury poisoning (Aaseth et al., 1995). Some physicians and dentists advocate a so-called DMPS challenge test in patients with symptoms attributed to amalgam (Godfrey and Campbell, 1994; Stenman and Grans, 1997). The patient gives a urine spot sample before taking the medication (usually in the form of a capsule) and gives another urine spot sample 1–2 h later. The increased concentrations are then assessed according to the standard limits for spot samples. We have previously shown that it is not possible to differentiate between patients with and without complaints self-related to their amalgam fillings using this test (Vannes et al., 2000).

Blood is not commonly used in the test. A study on humans given 300 mg DMPS orally revealed no changes in the blood mercury concentrations (Schuurs et al., 2000). We found no other information in the literature about any changes following low doses of DMPS given intravenously. It is not known to what extent potential changes reflect the patient’s symptoms or how they would correspond with changes in the mercury concentrations in urine.

The aim of this study is thus to determine whether the blood mercury levels after DMPS injection could assist in distinguishing between groups with and without symptoms self related to dental amalgams. Furthermore, it is our aim to investigate if there is a connection between a change in B-Hg and the urinary excretion of mercury during the first 30 min following DMPS injection.

2. Material and methods

The Regional Ethical Committee and the Norwegian Medicines Control Authority approved the present study. Written informed consent was obtained from all participants. Subjects, sampling and analytical procedure have been described in detail earlier (Vannes et al., 2000). Data from patients with complete records from blood and urine sampling are used in this study. A summary is presented here:

2.1. Subjects

A total of 80 individuals divided into four groups were investigated (Table 1):

1. Nineteen healthy non-patients without amalgam experience.
2. Twenty-one healthy non-patients with amalgam fillings.
Table 1
Age, gender and amalgam surfaces in the different groups of persons

<table>
<thead>
<tr>
<th>Patient category</th>
<th>Median age (min/max)</th>
<th>Male/female</th>
<th>Median Amalgam Surfaces (upper/lower quartiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Never had amalgam</td>
<td>21 (19/25)</td>
<td>10/9</td>
<td>0</td>
</tr>
<tr>
<td>2. Healthy with amalgam</td>
<td>43 (28/58)</td>
<td>6/14</td>
<td>43 (29–49)</td>
</tr>
<tr>
<td>3. Mercury alleged symptoms</td>
<td>46 (25/65)</td>
<td>5/13</td>
<td>37.5 (26–47)</td>
</tr>
<tr>
<td>4. Removed amalgam fillings</td>
<td>48 (24/65)</td>
<td>8/9</td>
<td>48 (23–58) (previous, now replaced)</td>
</tr>
</tbody>
</table>

3. Twenty patients with symptoms allegedly caused by their dental amalgam fillings.
4. Twenty patients who had removed their fillings because of concern about the safety of amalgam. The median time since removal of amalgam was 31.5 months (range 12–96 months).

The subjects in groups 1 and 2 were volunteers, while all other persons were recruited from the Dental Biomaterials Adverse Reaction Unit at the University of Bergen.

The exclusion criteria were occupational exposure to mercury, other heavy metals, or solvents; abuse of alcohol or drugs; or high consumption of seafood.

2.2. DMPS administration and blood sampling

An intravenous line was established in the forearm. DMPS (Heyl, Berlin, Germany) (2 mg/kg body wt.) was injected over a period of 5 min. Simultaneously, 500 ml Ringer’s acetate (Pharmacia, Stockholm, Sweden) was infused during 15 min. The line with Ringer’s acetate was established in order to maintain the intravenous access in case of allergic reactions or other emergencies and to provide volume so that there would be sufficient diuresis. Blood samples were drawn on sodium-heparinized tubes (Vacutainer R, Becton Dickinson Vacutainer Systems Europe, France) before infusion and injection was started, and 15 min, 30 min, 120 min and 24 h after start of injection. The samples were stored in acid-cleaned polypropylene tubes and frozen (−20 °C) until analyzed.

Since the infusion of Ringer’s acetate causes a dilution, we performed a control study on seven individuals. These were only given the Ringer’s solution, and the iron concentrations were measured in the same time course. Iron was chosen because it is a robust variable that could be easily and accurately measured. Iron concentrations in blood (B-Fe) were measured by means of flame atomic absorption spectrophotometry (Perkin–Elmer Aanalyst 800, Bodenseewerk, Überlingen, Germany). In this test, B-Fe fell 5.5% (median value), measured at the end of infusion of 500 ml Ringer’s acetate. This fluid disappears rapidly from the circulation through renal excretion and transport into the interstitial compartment. A numerical correction for this dilution would involve several uncertainties and was therefore not done (see Section 4).

2.3. Urine sampling

Emptying of the urinary bladder was performed prior to drug administration and 30 min after start of DMPS injection. The urine samples were collected in acid-cleaned polypropylene bottles and frozen (−20 °C) until processed.

2.4. Analytical procedure

Blood was digested by the microwave technique (Milestone 1200 MEGA, Sorisole, Italy). Analysis
of total mercury was done by cold vapor atomic absorption spectrophotometry (Perkin–Elmer FIMS 100, Bodenseewerk, Germany).

2.5. Statistics

All values are given in μg Hg/l. The group data are presented as medians and quartiles. The Wilcoxon test was used for paired two-sample statistics, and the Mann–Whitney U-test for independent pairs. The Kruskal–Wallis test was applied for multi-sample statistics. The Spearman rank test was used for testing of correlations between variables (r). A P-value less than or equal to 0.05 was considered statistically significant. Statistical software SPSS® 6.0 (SPSS Inc., Chicago, USA) was used for the calculations.

3. Results

The median mercury concentration in blood (B-Hg) before DMPS injection (base values) for the four study groups varied from 2.51 to 5.52 μg Hg/l (Fig. 1). The Kruskal–Wallis test revealed that the B-Hg differed significantly among the groups (P < 0.001). The subjects without amalgam experience had the lowest median B-Hg at all times. If this group was removed from the Kruskal–Wallis test, no statistical difference was found for the three remaining groups.

All study groups showed a statistically significant drop in B-Hg between base and lowest concentrations after the injection of DMPS (the Wilcoxon test) (Fig. 1). The drop in absolute concentration was smallest for the study group without amalgam experience (Group 1). In the group with amalgam alleged symptoms, the lowest median value occurred after 30 min; for the others, 15 min after injection. Cross-tabulations of the instances for the lowest values for all participants did not show statistically significant differences across the groups (P = 0.3). The drop in B-Hg relative to the base values varied between 24 and 30%, being statistically non-significant across the study groups (the Kruskal–Wallis test).

The median volume of urine for all groups was 100 ml after 30 min. There was no difference in the amounts of mercury excreted between the two groups with amalgam fillings (groups 2 and 3) or between the groups without amalgam fillings (groups 1 and 4) (Mann–Whitney U-test, P > 0.95). Following DMPS administration, the subjects with amalgam fillings (groups 2 and 3
Fig. 2. Dot plots of urinary mercury excretion during the first 30 min against blood mercury concentrations 30 min after injection of DMPS.

Drop in B-Hg can also be expressed as loss of mercury from blood. This loss in micrograms is calculated from changes in B-Hg and blood volume (Table 2). The blood volume in liter is set as 1/13 or 1/15 of bodyweight (in kg) in males and females, respectively (Witzleb, 1993). The esti-

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Never had amalgam</th>
<th>Removed amalgam</th>
<th>Healthy with amalgam</th>
<th>Alleged sympt. from amalgam</th>
<th>Without amalgam, pooled</th>
<th>With amalgam, pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated loss of B-Hg (µg Hg)</td>
<td>3.3 (1.8–6.3)</td>
<td>5.7 (3.2–8.5)</td>
<td>7.1 (4.5–10.5)</td>
<td>7.2 (3.8–10.7)</td>
<td>4.7 (1.8–7.3)</td>
<td>7.1 (4.1–10.6)</td>
</tr>
<tr>
<td>n = 19</td>
<td>n = 20</td>
<td>n = 18</td>
<td>n = 17</td>
<td>n = 39 P &lt; 0.05</td>
<td>n = 35</td>
<td></td>
</tr>
<tr>
<td>Urinary excretion 0–30 min (µg Hg)</td>
<td>2.8 (1.8–4.1)</td>
<td>3.7 (0.07–6.4)</td>
<td>10.7 (4.4–18.2)</td>
<td>9.8 (4.3–17.4)</td>
<td>3.1 (1.2–4.3)</td>
<td>10.7 (4.5–8.3)</td>
</tr>
<tr>
<td>n = 18</td>
<td>n = 18</td>
<td>n = 18</td>
<td>n = 13</td>
<td>n = 36 P &lt; 0.001</td>
<td>n = 31</td>
<td></td>
</tr>
</tbody>
</table>

Median values (lower to upper quartiles). Statistically significant differences between groups are shown where found.
mated loss of mercury in blood was higher than the corresponding excretion values in the pooled groups without dental amalgam, whereas the opposite was true for those with amalgam fillings. No differences were found when comparing each of the four groups separately.

4. Discussion

In humans, DMPS is cleared from the body primarily by oxidation to disulfide forms. When given intravenously, at least 80% is oxidized within the first 30 min (Maiorino et al., 1996). Within 96 h, approximately 84% of total DMPS is excreted the renal way (Maiorino et al., 1991). DMPS, when measured as a total drug, is eliminated from the body far more slowly than the unaltered form (Hurlbut et al., 1994). Unaltered DMPS does not enter red blood cells, but disulfide metabolites do. The ability of DMPS-metabolites to chelate metals is uncertain (Maiorino et al., 1991; Hurlbut et al., 1994; Islinger et al., 2001).

We observed an instantaneous, temporary drop in B-Hg 15–30 min after injection of the drug. Such a drop has not been described before, according to the literature available to us. Part of this drop is caused by dilution, as shown by the control study. Intravenously administered isotone saline solutions, e.g. Ringer’s acetate, are mainly distributed into the interstitial body fluid space. Only a small part remains in circulation (Lamke and Liljedahl, 1976; Calver et al., 1992; Tollofsrud et al., 2001). We gave 500 ml of Ringer’s acetate during 15 min. It has been shown that an infusion of 1000 ml of saline over 25 min to human volunteers caused plasma volume to increase by approximately 7% (Calver et al., 1992). Our control study on seven individuals indicated a blood volume increase of 5.5%, which is comparable to that found in the above mentioned studies. There was no significant difference between the four groups regarding the reduction in B-Hg when this is expressed as a fraction of the base values. This poses the question of the source for the mercury being chelated. If the DMPS available for chelating was the limiting factor, one would assume that there were smaller differences in the absolute reductions of mercury than what was actually found. Since the relative drop in B-Hg was similar (24–30%) in all the groups, one could speculate that there is a certain mercury fraction available for the initial chelation. Mercury in the body is mainly present in inorganic (Hg^{2+}) or organic (mostly methylmercury) form. Approximately 40% of mercury in human whole blood is inorganic (Brunetto et al., 1999). Approximately 95% of the methylmercury in blood is in the erythrocytes (Kershaw et al., 1980), and both forms can be bound to DMPS (Wildenauer et al., 1982; Arnold et al., 1983). It has been shown with high doses of DMPS that the penetration of erythrocytes by the chemical is time- and concentration-dependant (Wildenauer et al., 1982). The observed rapid fall in B-Hg may indicate the chelation of easily accessible inorganic mercury in serum. A study on rats where DMPS 100 mg/kg were given intraperitoneally, showed a significant drop of methylmercury levels in blood after 24 h (Pingree et al., 2001). Mass balance calculations indicated that the total amount of mercury excreted in the urine following DMPS injections corresponded quantitatively to the total amount of mercury removed from kidney, brain and blood. However, the study was done with rats exposed to organic mercury, a 50-fold greater dosage of DMPS, and the samples were taken after 24 h. In the present low dose study on humans, organic and inorganic mercury was not speciated.

The drop we found in B-Hg during the first 30 min, corresponds to a blood mercury loss of at least 70% of the urinary mercury excretion in the same period. Other authors have pointed out that the administration of DMPS clearly reduces the renal mercury burden, and that the excreted mercury originates from the kidneys (Zalups, 1993). In our opinion, the rapid drop of B-Hg and corresponding urinary mercury excretion indicates that at least some of the excreted mercury might originate from circulating blood—most likely serum. The B-Hg levels slowly rise and return to base value levels 2–24 h after the DMPS injection, indicating redistribution. We have found earlier that the greatest urinary excretion of mercury takes place within the first 30 min after injection of DMPS (Vannes et al., 2000). Therefore, we did
not consider the mercury excretion after this first period.

The elimination of mercury from blood after amalgam removal has been described using a biexponential model with a half time of 75–88 days (Molin et al., 1990; Sandborgh-Englund et al., 1998). In the present study the B-Hg levels in the group with no amalgam experience (group 1) was significantly lower than in group 4, where the amalgam fillings had been removed 12–96 months ago. This is not according to the described half-life. The median age in group 1 was 21 years, in group 4 it was 48 years. The median age in group 1 reflects the reduction of caries in the Norwegian population—it was not possible to find an age matching group which had never had dental amalgams. The difference in B-Hg between the groups may be explained as a mercury accumulation with aging (Weiner and Nylander, 1993). Concerning drug metabolism, groups 1 and 4 are considered equal as age-related changes in pharmacokinetics and pharmacodynamics does not occur below the age of 55 (Plein and Plein, 1984).

When related to the estimated loss of mercury in blood, the groups with dental amalgam had a higher Hg-excretion than the other groups. This might be explained by the higher concentration of mercury in the kidney cortex in amalgam carriers (Nylander et al., 1989; Hultman et al., 1994) and additional chelation in the excretion period of DMPS. It is also possible that oxidized DMPS is reduced to the active form when it enters the renal tubular cells (Islinger et al., 2001).

As in other studies (Nylander et al., 1987; Langworth et al., 1988; Oskarsson et al., 1996) there is a great scatter of data in our results (Figs. 1 and 2).

In conclusion, the intravenous injection of DMPS causes an instantaneous but temporary drop of 24–30% in B-Hg levels in all groups of subjects. The change in values of B-Hg does not differentiate between patients with and without self-reported symptoms from dental amalgam fillings. There are indications that part of the urinary mercury excreted during the first 30 min originates from the blood.

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References


Maiorino RM, Xu ZF, Aposhian HV. Determination and metabolism of dithiol chelating agents. XVII. In humans, sodium 2,3-dimercaptopropane-1-sulfonate is bound to plasma albumin via mixed disulfide formation and is found in the urine as cyclic polymeric disulfides. J Pharmacol Exp Ther 1996;277:375–384.


Zalups RK. Influence of 2,3-dimercaptopropane-1-sulfonate (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA) on the renal disposition of mercury in normal and uninephrectomized rats exposed to inorganic mercury. J Pharmacol Exp Ther 1993;267:791–800.