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Effect of dental amalgam on gene expression profiles in rat cerebrum, cerebellum, liver and kidney

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ABSTRACT — Dental amalgam is a source of exposure to elemental mercury vapor in the general population. The aim of this study was to elucidate the effect of elemental mercury vapor exposure from dental amalgam restorations on gene expression profiles. Out of 26,962 rat genes, mercury vapor was found to increase the expression of 1 gene (*Atp1b3*) and decrease the expression of 1 gene (*Tap1*) in the cerebrum, increase the expression of 1 gene (*Dnaja2*) in the cerebellum, increase the expression of 2 genes (*Actb* and *Timm23*) and decrease the expression of 1 gene (*Spink3*) in the liver, increase the expression of 2 genes (*RT1-Bb* and *Mgat5*) and decrease the expression of 6 genes (*Tnfaip8, Rara*, *Slc2a4, Wdr12, Pias4* and *Timm13*) in the kidney.

Key words: Dental amalgam, Mercury vapor, Rat tissue, Gene expression profiles

INTRODUCTION

Dental amalgam was introduced more than 150 years ago as a tooth filling restoration and is generally the major source of mercury vapor exposure (WHO, 1991). Many studies have examined the relationship between dental amalgam fillings and the amount of mercury in organs, blood, urine and other tissues. In our previous experiment, we also found a mercury uptake in maternal and fetal tissues from the maternal amalgam fillings and that the amount of mercury vapor exhaled by the rat increased 7-20-fold after chewing (Takahashi *et al*., 2001). However, adverse health effects from mercury accumulated in the tissues were not clear. In the present study, to elucidate the effect of elemental mercury vapor exposure on the gene expression profiles, we examined the gene expression associated with dental amalgam fillings in rat tissues using DNA microarray analysis.

MATERIALS AND METHODS

Animal experiment and amalgam fillings

Thirty-five 10-week-old female rats of the Sprague-Dawley strain were purchased from Japan SLC Inc. (Shizuoka, Japan). Twenty-five rats were used as experimental animals, while ten rats were employed as controls. Experimental groups received 4 amalgam fillings (both first and second molars in the maxilla) under anesthesia (NEMBUTAL® 50 mg/ml, 0.05 ml per 100 g). Dental procedures were those employed previously (Takahashi *et al*., 2001, 2003). The rats from control and experimental groups were further divided into smaller groups of two or three for housing in cages for six months. The cages were suspended over a rack regularly flushed clean of rat droppings with tap water. All animals were fed with a commercial pellet diet (Nippon Clea Co., CE-2, Tokyo, Japan). The diet and tap water was given *ad libitum*. All animals were maintained in an environmentally controlled room at 23 ± 1°C, with a 12 hr light: 12 hr dark cycle. They received humane care throughout

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the experiment according to the guidelines established by the Aichi Gakuin University. Six months later, all animals were given an anesthetic dose of sodium pentobarbital and were positioned on the operating pad. In the experimental group, two rats died before the operation began, reducing the number of animals in this group to 23. Anesthesia was the cause of death. The animals were terminated by bleeding from the right auricle of the heart and were then transcardially perfused with normal saline solution for 1 hr. After perfusion, cerebrum, cerebellum, liver and kidney were removed, rinsed with saline solution and blotted with filter paper. Finally, the maxilla of each rat in the experimental group was removed to examine the surviving amalgam fillings. All amalgam fillings showed signs of wear after six months. Two rats had lost all 4 amalgam fillings after six months, 4 rats had lost 3 amalgam fillings each, 7 rats had lost 2 amalgam fillings each, 7 rats had lost 1 amalgam filling each, and 3 rats still had all 4 amalgam fillings. For DNA microarray analysis, three rats having 4 amalgam fillings and 2 rats having 3 amalgam fillings were chosen from the experimental groups, while 5 rats from the control group were chosen randomly.

Preparation of total RNA

Total RNA was extracted from the cerebrum, cerebellum, liver and kidney in each group using the Quick Gene RNA tissue kit SII (Fujifilm, Tokyo, Japan) according to the manufacturer's protocol and stored at -80°C.

DNA microarray analysis

DNA microarray analysis was described previously (Fujiwara *et al*., 2011). Briefly, purified total RNA (5 μg) was applied to an OpArray™ Rat V3.0 slide that had 26,962 genes registered (Operon Technologies, Alameda, CA, USA). The Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies, Santa Clara, CA, USA) was used to synthesize complementary RNA (cRNA) from the double-stranded cDNA template. A primer containing poly dT and the T7 polymerase promoter was annealed to poly A^+ RNA. Reverse transcriptase was then added to the reaction mixture to synthesize the first and second strands of cDNA. Next, double-stranded cDNA from control and experimental groups was transcribed in the presence of cyanine (Cy) 3 and Cy5-labeled nucleotide, respectively. These two sets of fluorescently-labeled cRNA were mixed and hybridized to an OpArray™ slide for 16 hr at 42°C using a Lucidea SlidePro Hybridizer (GE Healthcare, Buckinghamshire, England). A fluorescent image of the slide was recorded with CRBIO (Hitachi Software Engineering, Tokyo, Japan). Digitized image data were processed with DNA-SIS Array software (Hitachi Software Engineering). After global normalization, the data were filtered to exclude genes with low expression levels. The ratio of the intensity of Cy5 (experimental group) to that of Cy3 (control group) was calculated. Genes with an expression level change greater than 2-fold and less than 0.5-fold were used to select up- or down-regulated genes, respectively. Information on each gene on the slide was obtained from the National Center for Biotechnology Information (NCBI) database.

Mercury accumulation in tissues

Total mercury concentrations in organs were determined by the oxygen combustion-gold amalgamation method (Ohkawa *et al*., 1977) using an atomic absorption mercury detector MD-A (Nippon Instruments, Co., Ltd., Osaka, Japan).

Statistical analysis

The Mann-Whitney test was used for statistical analysis.

RESULTS AND DISCUSSION

To our knowledge, this is the first report to examine the relationship between dental amalgam fillings and gene expression associated with dental amalgam fillings in rat tissues using DNA microarray analysis. Out of 26,962 rat genes, mercury vapor was found to increase the expression of 1 gene (*Atp1b3*) and decrease the expression of 1 gene (*Tap1*) in the cerebrum (Table 1), increase the expression of 1 gene (*Dnaja2*) in the cerebellum (Table 2), increase the expression of 2 genes (*Actb* and *Timm23*) and decrease the expression of 1 gene (*Spink3*) in the liver (Table 3), increase the expression of 2 genes (*RT1-Bb* and *Mgat5*) and decrease the expression of 6 genes (*Tnfaip8, Rara*, *Slc2a4, Wdr12, Pias4* and *Timm13*) in the kidney (Table 4).

Although experimental methods and target organs are partly different from the present study, there are some studies of animals exposed to mercury vapor (Brambila *et al*., 2003; Liu *et al*., 2003; Yoshida *et al*., 2011). However, we could not find the same change of gene expression in organs such as the brain, lung and kidney.

The amounts of mercury in the cerebrum, cerebellum, liver and kidney in the experimental groups were higher than those of the controls and significant ($p < 0.05$) or highly significant ($p < 0.01$) differences were observed between them (Table 5). Results of the present study showed that mercury vapor release affected the genes in the organs of rats which received dental amalgam fillings,

Table 1. Changes in gene expression in rat cerebrum after exposed to mercury

Gene name	Accession number	Fold change
Up-regulated genes (> 2.0-fold)		
1. *ATPase, Na+/K+ transporting, beta 3 polypeptide* (*Atp1b3*)	NM_012913	2.12
Down-regulated genes (< 0.5-fold)		
1. *transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (Tap1)*	NM_032055	0.47

The genes for which the levels of expression changed more than 2-fold are listed here.

Table 2. Changes in gene expression in rat cerebellum after exposed to mercury

Gene name	Accession number	Fold change
Up-regulated genes (> 2.0-fold)		
1. *DnaJ (Hsp40) homolog, subfamily A, member 2* (*Dnaja2*)	NM_032079	2.05
Down-regulated genes (< 0.5-fold)		
None		

The genes for which the levels of expression changed more than 2-fold are listed here.

Table 3. Changes in gene expression in rat liver after exposed to mercury

Gene name	Accession number	Fold change
Up-regulated genes (> 2.0-fold)		
1. *actin, beta (Actb)*	NM_031144	2.38
2. *translocase of inner mitochondrial membrane 23 homolog (yeast) (Timm23)*	NM_019352	2.12
Down-regulated genes (< 0.5-fold)		
1. *serine peptidase inhibitor, Kazal type 3 (Spink3)*	NM_012674	0.45

The genes for which the levels of expression changed more than 2-fold are listed here.

Table 4. Changes in gene expression in rat kidney after exposed to mercury

Gene name	Accession number	Fold change
Up-regulated genes (> 2.0-fold)		
1. *RT1 class II, locus Bb (RT1-Bb)*	NM_001004084	2.71
2. *mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase* (*Mgat5*)	NM_023095	2.04
Down-regulated genes (< 0.5-fold)		
1. *tumor necrosis factor, alpha-induced protein 8 (Tnfaip8)*	NM_001107387	0.28
2. *retinoic acid receptor, alpha (Rara)*	NM_031528	0.35
3. *solute carrier family 2 (facilitated glucose transporter), member 4 (Slc2a4)*	NM_012751	0.47
4. *WD repeat domain 12 (Wdr12)*	NM_199410	0.48
5. *protein inhibitor of activated STAT, 4 (Pias4*)	NM_001100757	0.48
6. *translocase of inner mitochondrial membrane 13 homolog (yeast) (Timm13)*	NM_145781	0.49

The genes for which the levels of expression changed more than 2-fold are listed here.

Table 5. Mercury concentrations in major organs (ng/g tissue)

	Cerebrum	Cerebellum	liver	kidney
Control group (n = 5)	7.5 ± 2.3[a]	6.8 ± 3.3[a]	19.8 ± 4.5[b]	367.3 ± 75.9[b]
Experimental group (n = 5)	12.4 ± 3.6[a]	11.8 ± 3.0[a]	34.9 ± 7.6[b]	5765.2 ± 2480.4[b]

[a] Statistically significant in $P < 0.05$.
[b] Statistically significant in $P < 0.01$.

and will be helpful for further experiment on the relationship between dental amalgam fillings and adverse health effects.

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