IMMUNOLOGICAL AND BRAIN MRI CHANGES IN PATIENTS WITH SUSPECTED METAL INTOXICATION

LITA TIBBLING,* KARL-ÅKE THUOMAS,† RODRICA LENKEI,‡ AND VERA STEJSKAL§

*Department of Otorhinolaryngology
†Department of Diagnostic Radiology
University Hospital
Linköping, Sweden

‡CALAB Medical Laboratories
Stockholm, Sweden

§AB Astra Safety Assessment
Södertälje, Sweden

Thirty-four patients with central nervous system (CNS) and systemic symptoms suggestive of intoxication from dental amalgam were examined with magnetic resonance imaging (MRI) of the brain (n = 32) and with a Memory Lymphocyte Immuno Stimulation Assay (MELISA) (n = 17). Lymphocyte phenotype was analyzed with flow cytometry (FC) in 22 of the patients. One hundred twenty age-matched patients without CNS symptoms served as controls for the MRI study, seventy-seven healthy subjects with dental amalgam fillings served as controls for the MELISA test, and seventy-five clinically healthy subjects were controls for lymphocyte phenotype determination. Pathological MRI findings were present in 81% of the patients, most of them with signs of degeneration in the basal ganglia, but in none of the controls. The lymphocyte phenotype determination was pathological in 58%. The MELISA showed pathological findings in 88%, of which 60% showed an immune reaction to mercuric chloride, 62% of the patients had some kind of atopic disease, and 35% suffered from levothyroxine-treated hypothyreosis. A high rate of immunopathologies and objective signs of immunological reactions in the majority of the patients

1. Address all correspondence to: Dr. Lita Tibbling, M.D., Ph.D., Department of Otorhinolaryngology, University Hospital, S-581 85 Linköping, SWEDEN. Tel.: +46-13-222552. Fax: +46-13-145949.

2. Abbreviations: CNS, central nervous system; FC, flow cytometry; FITC, Fluorescein Isothiocyanate; FSE, fast spin–echo; MAbs, monoclonal antibodies; MRI, magnetic resonance imaging; MELISA, Memory Lymphocyte Immuno-Stimulation Assay; PE, Phycoerythrin, SE, spin-echo.

3. Key Words: amalgam, basal ganglia, immunopathologies, magnetic resonance imaging, mercury, white matter.

International Journal of Occupational Medicine and Toxicology, Vol. 4, No. 2, pp. 285–294

ISSN: 1054-044X

with MRI changes in the brain suggests that immunological mechanisms may play an important role in the development of the lesions.

INTRODUCTION

Dental amalgam, which is composed of metallic mercury, silver, copper, tin, and zinc, has been the subject of intermittent controversy since it was introduced into dental practice 150 years ago. When they interact with saliva, amalgam fillings will slowly corrode (Brune et al., 1983; Brune and Evje, 1985). Radioactive ^{203}Hg (Hahn et al., 1989, 1990) and radiochemical methods (Eggleston and Nylander, 1987; Weiner and Nylander, 1993) have shown deposition of mercury in several body tissues and in the brain.

Patients with dental amalgam may suffer from various symptoms of central nervous system (CNS) disorders (D'Itri), as well as symptoms indicating some immune disorder. Dentists with high mercury levels in their tissues have significantly higher rates of polyneuropathies and mild neuropsychological impairments than controls (Shapiro et al., 1982; Ngim et al., 1992). Patients who suffered from the Minamata disease in 1953 and who were reexamined three decades later had symptoms similar to the ones found in patients with chronic amalgam intoxication, e.g., tremor, stumbling, forgetfulness, fatigue, weakness, dizziness, and tinnitus (Kinjo et al., 1993).

Magnetic resonance imaging (MRI) of the CNS gives *in vivo* information on different kinds of tissue damages as well as on certain chemical depositions. Different metals from amalgam and other dental restorations can accumulate in the brain, causing alterations of lymphocyte phenotype, as has been found in experimental studies (Bigazzi, 1992) and in observations of patients with symptoms suspected to be due to amalgam intoxication (Eggleston, 1984). Furthermore, it is known that the immune system is involved in brain pathology (Reichlin, 1993).

The aim of this study was to investigate patients who suspected chronic amalgam intoxication as a cause of their symptoms. This was done with MRI of the brain, with tests of immunological reactivity to metals as determined by Memory Lymphocyte Immuno-Stimulation Assay (MELISA), and with lymphocyte phenotyping. Finally, these patients were compared with symptom-free controls.

SUBJECTS AND METHODS

The study was comprised of 34 patients (24–75 years of age; 25 women and 9 men). Due to suspicions of chronic mercury intoxication from dental amalgam, these patients had been referred consecutively to an ear-nose-throat clinic. All patients suffered from symptoms indicating CNS involvement, such as headache, dizziness, insomnia, nervous irritability, tinnitus, visual disturbances, memory impairment, inability to concentrate, mood swings, and pronounced fatigue. They had been extensively investigated by neurologists, internists, and

some even by specialists in infectious diseases, but no diagnoses had been found that could explain their CNS symptoms. Some kind of atopic disease (asthma, hay fever, eczema) was present in 21 patients. Twelve patients were being treated for hypothyreosis. One female patient had Sjögren's syndrome, and another had glomerulonephritis.

Controls

A matched control group of 120 healthy volunteers (22–71 years of age) underwent the same MRI investigation of the brain as the patients. The volunteers had no CNS symptoms, but they had amalgam fillings and often other metallic restorations such as golden bridges and crowns. In Sweden, an adult has an average of 14 amalgam fillings in his or her teeth. Seventy-seven healthy subjects (20–65 years of age) served as controls for MELISA, and another group of seventy-five clinically healthy subjects (age range 18–58 years; 40 men and 35 women) served as controls for the lymphocyte phenotype determination. All these control subjects had metal dental fillings.

MRI Studies

All patients and controls were examined with superconductive 1.5 T-equipment using SE sequences and an FSE sequence. A Helmholtz head coil served as transmitter and receiver. Axial and coronal SE images with T_1 weighting and proton/T_2 weighting were acquired with a 24-cm field of view, 3-mm slice thickness with 10% interslice gap, and a 256 x 256 acquisition matrix. The images covered the whole brain.

Single slices which showed most of the basal ganglia in the coronal and axial projections were chosen. Single-slice, single-echo SE sequences and a single-slice, multiple-echo SE sequence with eight equidistant echoes, as well as an FSE sequence with eight-echo Carr-Purcell-Meiboom-Gill (CPMG) train, were applied at these positions.

T_1 was determined from the above three single-slice, single-echo SE sequences, while the transverse relaxation time, T_2, was measured by the CPMG method of pulse sequences (Farrar and Becker, 1971); T_2-values were taken from T_2 maps. In order to assess T_2 changes in the basal ganglia on MRI, 15 pixel regions of interest (ROI) were studied. The imaging parameters provided a pixel approximately 0.3 mm x 0.3 mm in the imaging plane. T_1- and T_2-values were also determined in gray and white matter in the frontal, temporal, parietal, and occipital lobes, and in the brain stem. All sequences used are standard and commercially available.

In all MRI examinations, two syringes containing 10 mmol/l aqueous solutions of copper sulfate were used as references for the relaxation time measurements. These solutions constantly gave monocompartment T_1 and T_2, consistent over time. One of the syringes was placed under the head in the midsagittal plane and the other close to the right temporal lobe. The syringes were fixed to the head coil.

The MRI examinations were analyzed in three steps: intensity changes in gray and white matter on the images, T_1 and T_2 estimations, and cortical atrophy. The intensities in the SE and FSE images of the patients were compared with the control group. On SE and FSE images, the intensities were graded in four levels: absent, slight, mild, and moderate changes. The degrees of cortical atrophy were: absent, slight, mild, and moderate.

The MELISA is based on the idea that cultivation of lymphocyte populations containing memory cells, together with the specific antigen, will result in stimulation and proliferation of T-cells (Stejskal et al., 1994). One million peripheral blood lymphocytes per ml per well were incubated for five days in 48 well plates with various metal salts. At the end of the cultivation, H^3-thymidine was added to the cultivation chambers for four hours and incorporated into the nuclei of dividing cells. The DNA radioactivity was measured by a beta-counter which gives an objective measure of cell proliferation. Since even macrophages may divide *in vitro* and thus cause false-positive results, the presence of lymphoblasts in stimulated cultures was also evaluated under microscope. A stimulation index (SI) greater than three was regarded as a positive immune response.

Lymphocyte Phenotyping with FC

Blood samples were obtained from the first 24 patients. Lymphocyte populations were analyzed by FC with dual-color direct immunofluorescence after whole-blood lysis (Lenkei et al., 1991). The samples were analyzed with a FACStar flow cytometer (Beckon Dickinson, Immunocytometry Systems, San José, California), which was calibrated daily with polystyrene beads (Calibrite, Beckon Dickinson, Mountain View, California). All monoclonal antibodies used to detect the lymphocyte subsets presented in Table 1 were purchased from Beckon Dickinson. Samples from patients and controls were stained according to the same protocol and method. For each parameter, we evaluated the number of patients who presented values outside the 95% confidence interval calculated on the basis of the data obtained from studies of the control group.

TABLE 1. Staining Protocol for Lymphocyte Phenotyping

	MAbs conjugated with:		
Tube	FITC	PE	Population detected
1	IgG_1	IgG_{2a}	Isotype control
2	CD_{45}	CD_{14}	All lymphocytes, monocytes
3	CD_{20}	CD_5	All B-cells, B-cell subset
4	CD_{20}	CD_{23}	All B-cells, activated B-cells
5	HLA-DR	CD_4	All T-helper, T-helper activated
6	HLA-DR	CD_8	All T-supp. cytotox., activated cells
7	CD_8	CD_{11b}	Suppressor T-lymphocytes
8	CD_3	CD_{16} & CD_{56}	NK cell subsets
9	CD_{57}	CD_{56}	NK cell subsets

Statistical Evaluation

The difference between the groups in MELISA was evaluated using Fisher's exact test (two-sided). The significance of lymphocyte subset alterations was also calculated with Fisher's exact test. A p-value of less than 0.05 was regarded as significant, while $p = 0.05$ was regarded as weakly significant.

RESULTS

Findings on brain MRI and immunological tests in each patient are presented in Table 2.

Neuropathological changes on MRI were seen in 26 patients, but in none of the controls. Twenty-three of the patients showed a decreased signal in the basal ganglia; two of these patients also had a decreased signal in the caudate nuclei, and three patients had a decreased signal in the thalamus. In 12 of the patients, there was diffuse hyperintensity of the white matter with loss of the gray-white matter discrimination as a sign of demyelination. Intensity changes in the tissues were equal in the left and right side of the brain. Six of the patients showed no any intensity changes in gray or white matter.

In T_1 of the basal ganglia, there were no significant differences between patients and controls. The T_2-values were prolonged in patients with diffuse hyperintensity of the white matter, while they were shortened in patients with a decreased signal in the basal ganglia on T_2-weighted images. Because they show T_2 changes better than absolute values, the relative values of the relaxation times, and patient/control ratio, are shown (Table 3).

In a significant number of patients, lymphocyte phenotyping with FC revealed increases in the absolute numbers of B-lymphocytes (CD_{20}^+) and B-lymphocyte subpopulations $CD_{20}^+CD_5^+$ and $CD_{20}^+CD_{23}^+$ ($p < 0.02$). Among T-lymphocytes, an increase was observed in the number of cells with the suppressor phenotype $CD_8^+CD_{11b}^+$ ($p < 0.001$). A reduction in the percentage of cells expressing CD_{57} was also observed in a significant number of patients ($p < 0.01$). Although it did not reach statistical significance, there was a tendency towards an increase in the CD_4/CD_8 ratios and in the number of CD_4^+HLA-DR$^+$ T-cells, and a decline in the number of cells expressing CD_3 (all T-lymphocytes). Ten out of twelve patients with alterations in lymphocyte phenotype had some kind of atopic disease.

The MELISA showed a higher frequency in patients than in controls of memory cells for several metals (Table 4). The difference in metal reactivity was highly significant for inorganic Hg ($p < 0.001$), phenyl Hg ($p < 0.002$), and gold ($p < 0.005$), weakly significant for lead ($p = 0.05$), and not significant for remaining metals. Both in the patients and in the controls, the most frequent memory cells detected were for nickel.

TABLE 2. **Findings on Brain MRI and Immunological Tests in Each Patient**

Patient no.	MRI	MELISA	L.p.	Atopy	Hypothyreosis
1	+	+	+	+	
2	+	–	–	+	
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+		+	+
6		–			
7	+	–			+
8		–	–		
9		–	+		
10	+	–	+	+	+
11	+		–	+	+
12	+	+	+	+	+
13		–	–		
14	+	+			
15	+	+	–	+	
16	+	+	+		
17	+	+		+	
18	–	–	+		
19		–	+	+	
20	+	–	+	+	+
21	+	–		+	
22	+	–	–	+	
23	+	+	+	+	
24	+	–	+	+	
25		–		+	
26	+		–		
27	+	–	+		
28	–	–	+		
29	+	–			
30	+	+			
31	+	+		+	+
32	+	+	–	+	+
33	+	+	–	+	+
34	+	+	–	+	+
	26/32	15/17	14/24	21/34	12/34

+, pathological finding; –, not performed; L.p., lymphocyte phenotyping (at least one marker presenting values outside the 95% confidence interval).

DISCUSSION

The current study shows a significant correlation between MRI findings in the brain and CNS symptoms resembling chronic mercury intoxication. Furthermore, patients with degenerative changes in gray and white matter had a high rate of immunological diseases such as thyroiditis, as well as objective findings of T-lymphocyte alterations and increased frequency of metal-specific lymphocytes.

TABLE 3. MRI Abnormalities Accounted for As Relative T_2-Values in 32 Patients

MRI abnormalities in T_2-weighted images	Relative T_2-values (T_{2pat}/T_{2con}) + SD			
	White matter	Putamen	Thalamus nucleus	Caudate nucleus
slight	1.07 + 0.02	0.95 + 0.02	0.97 + 0.02	0.97 + 0.02
mild	1.15 + 0.03	0.91 + 0.03	0.94 + 0.02	0.93 + 0.02
moderate	1.25 + 0.03	0.85 + 0.03		

pat = patients, con = control group of healthy volunteers.

TABLE 4. Number of Positive (# pos) MELISA in Patients (n = 15) and Controls (n = 77), Percent Positive Results Shown in Parentheses

Metal in culture	Controls: # pos/total	Patients: # pos/total	*P*-value
$HgCl_2$	5/71 (7)	9/15 (60)	< 0.001
Phenyl Hg	7/72 (10)	7/15 (47)	< 0.002
Sn	4/49 (8)	2/15 (13)	n. s.
Au	2/51 (4)	5/15 (33)	< 0.005
Pd	7/46 (15)	4/15 (27)	n. s.
Pb	3/47 (6)	4/15 (27)	= 0.05
Cd	10/47 (21)	5/15 (33)	n. s.
Ti	4/39 (10)	2/15 (13)	n. s.
Methyl Hg	2/39 (5)	3/15 (20)	n. s.
Ni	18/42 (42)	10/15 (66)	n. s.

These findings give rise to two major questions: Can metals be responsible for the MRI changes? Why are MRI changes in the basal ganglia and the white matter found only in patients with CNS symptoms and not in the control group, which is comprised of people with a similar metal load in the oral cavity?

In a recent study, Schenker et al. (1993) demonstrated that changes in signal intensity in the basal ganglia on T_2-weighted MRI are very likely to be produced by iron and that the curves of T_2 relaxation times in the basal ganglia are congruent with published curves of age-dependent iron concentration (Hallgren and Sourander, 1958). This was taken into consideration in the present study through the comparison of patients with age-matched controls. Copper is known to accumulate in the basal ganglia in Wilson's disease, which gives the same kind of hypointensive signals on MRI in the putamen as were found in our patients.

Copper, manganese, and particularly iron, are cofactors needed for the normal metabolic activity of the basal ganglia. However, these metals can be involved in deleterious biochemical reactions, such as the production of destructive oxygen-derived free radicals (Ikeda and Long, 1990). It is well known that certain metals such as copper, a component of copper amalgam, interfere with mitochondrial enzymes.

Thus, the deposition of several metals may contribute to MRI changes in the basal ganglia. Mercury and other metals used in restorative dentistry, e.g., gold, silver, and palladium, are not paramagnetic and therefore cannot be traced directly by MRI. However, theoretically, they could cause degeneration, and consequently T_2 shortening on MRI. By contrast, copper, nickel, and tin are paramagnetic and, under certain conditions, considerably toxic.

The CNS itself can be involved in immune reactions within the brain or in responses to peripheral immune stimuli (Reichlin, 1993). As a result of chemically induced breakdown of the blood-brain barrier (Chang and Hartmann, 1972), metallic corrosion products disseminated by phagocytic cells such as granulocytes and macrophages may penetrate the brain. In this case, not only mercury, but also other metals such as gold, palladium, and silver, which cannot be traced by MRI, may be stored in the brain. The interference of metals with intracellular mitochondria and enzyme systems may further aggravate the lesions in the brains of affected individuals.

The increased prevalence of metal-specific lymphocytes and the alteration in lymphocyte phenotyping observed in MRI-positive patients are compatible with possible immunopathologic processes in the brain or elsewhere in the body. For example, mercury and gold, which have a strong affinity to SH-groups in proteins, may be deposited in the brain and other tissues by scavenger macrophages. Consequently, the antigenicity of autologous proteins may be changed, triggering a local inflammatory reaction. Indeed, several patients in this study suffered from autoimmune diseases such as thyroiditis, Sjögren's syndrome, or glomerulonephritis.

Regardless of the metal or metals responsible, the question still remains why only certain subjects suffer from subjective and objective CNS manifestations. Chronic exposure of humans to low doses of metals has been shown to induce autoimmunity in subjects genetically predisposed to autoimmune disease (Bigazzi, 1988; Eneström and Hultman, 1995). Mercury- and gold-induced autoimmunity has been reviewed recently (Goldman et al., 1991) and could provide an explanation why only certain predisposed individuals developed CNS lesions and symptoms in this study.

In toluene abusers, Caldemeyer et al. (1993) have found diffuse cerebral, cerebellar, and brain-stem atrophy, poor differentiation of gray and white matter, increased periventricular signal intensity on T_2-weighted images, and hypointensity signals in the basal ganglia. They proposed that increased iron deposition might account for the hypointensity. However, Unger et al. (1994) claimed that iron deposition is not the dominant cause of regions of hypointensity in toluene abusers. They suggested that a partitioning of toluene into the lipid membranes of cells in cerebral tissue may be responsible for the hypointensity in the basal ganglia noted on T_2-weighted MR images of the brain.

Brautbar et al. (1994) studied the production of autoantibodies, the regulation of T- and B-cells, and the quantitative function of lymphocyte subsets and natural killer-cell activity in

289 subjects who had been exposed to organic solvents. They found a significant number of exposed individuals with symptomatology of the CNS, abnormal T_4/T_8 ratios, and higher levels of autoantibodies and immune complexes than in controls. This immune dysregulation caused by chemical solvents seems to be akin to that found in our patients. Thus, it seems likely that any xenobiotics, whether chemical solvents or metals, can induce immunopathologies involving the CNS as well as the peripheral organs. At present, it is not possible to differentiate between damages caused by solvents and the damages we observed in patients who were suspected to suffer from metal intoxication.

In conclusion, this study shows a strong correlation between neuropathological changes, T-lymphocyte pathology, and metal-specific lymphocytes in patients with symptoms resembling metal intoxication.

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