Rapid changes in concentrations of essential elements in organs of rats exposed to methylmercury chloride and mercuric chloride as shown by simultaneous multielemental analysis

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Abstract

An *in vivo* study of rats given a dominant lethal dose of methylmercury chloride (MMC) or mercuric chloride (HgCl_2) was conducted to elucidate the rapid biotransformation of essential elements. The elements were measured by inductively coupled plasma atomic emission spectrometry. For the rat brain Zn concentrations were higher in the MMC group than in the HgCl_2 and control groups. The highest Cu concentration was found in HgCl_2 dosed rat liver. For the rat kidney the highest Zn concentration was seen in the MMC group. From principal component analysis on the time dependent behaviour of each element in rat organs, characteristics specific to Cu in the liver and kidney and Mn in the brain were found after exposure to HgCl_2 , and Ca and Zn in the brain after exposure to MMC.

Previous studies have suggested that the toxic effects of methylmercury are a result of its ability to rapidly cross permeability barriers such as the blood brain barrier.¹ Inorganic mercury is also transported through lipid bilayer membranes.²

Monitoring the phase transition temperature and the permeability of phospholipid bilayer vesicles, Bevan *et al* reported that the relative effectiveness of the metal ions, in decreasing order, was $\text{Zn}^{2+} > \text{Cd}^{2+} > \text{Hg}^{2+} \gg \text{Ca}^{2+} > \text{Mg}^{2+}$. These interactions of Zn^{2+} , Cd^{2+} , and Hg^{2+} with phospholipids in membranes may be physiologically significant.³ It has also been found that association of mercury with metallothionein results in altered amounts of Zn and Cu.⁴⁻⁶ Furthermore, the addition of Hg^{2+} to loaded kidney mitochondria induces the fast release of accumulated

cation and the Ca^{2+} efflux exhibits kinetics that depend on the extent of the binding of Hg^{2+} to the membrane.⁷

On the other hand, membrane lipid peroxidation and free radical formation induced by mercury exposure cause damage to biological membranes.⁸⁻¹⁰ Of the many physiological effects of mercury, it has been shown to alter the metabolism of some of the essential elements including Zn.¹¹⁻¹⁴

We report here the rapid concentration changes of essential elements in rat organs after a dominant lethal dose of mercury.

Materials and methods

CHEMICALS

All chemicals were of reagent grade quality or provided specifically for trace element analysis and were obtained from Wako Pure Chemical Industries Ltd, Osaka, Japan. Standard stock solutions of Na, K, Mg, Ca, Fe, Mn, Al, Cu, Zn, and Hg were prepared from 1000 ppm certified atomic absorption reference solutions.

ANIMAL STUDIES

Male Wistar rats weighing 180 to 220 g were housed in stainless steel cages with five or six animals a cage. They were given unlimited commercial pellet diet and tap water for at least one week before use and during the study. Twelve rats were used for each of the methylmercury chloride (MMC) dosed, mercuric chloride (HgCl_2) dosed, and control groups. Each 1 ml of MMC (10 mg/kg b w) premixed with L-cysteine solution (3 mg/ml 0.9% saline), HgCl_2 (1 mg/kg bw) dissolved in 0.9% saline solution, or 0.9% saline solution alone (control) was injected subcutaneously into the rats by a single dose. Three rats were selected randomly from each group at three, six, 12, and 24 hours after injection and killed at these times. The brain, liver, and kidneys were excised, rinsed in distilled water, and homogenised at high speed in an ultrahomogeniser (Physcotron NS-50, Ikemoto Sci Technol Co Ltd, Japan) until a smooth homogenate was obtained. The homogenised sample, 1-10 g depending on the individual sample size, was weighed into a metal free

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100 ml beaker. In a chemical clean hood 1:1 concentrated $\text{HNO}_3/\text{HClO}_4$ (10 ml) was added to the sample and the beaker, covered with a watch glass, was placed on a hot plate (HK-41, Yamato Sci Co Ltd, Japan). The hot plate was maintained at 150°C and the contents of the beaker allowed to simmer stirring frequently with a glass rod. This avoided loss of sample by overfrothing. The solution was then taken down to near dryness indicated by foaming on the bottom of the beaker, 11.6% $\text{HCl}/2.8\%$ HNO_3 (3 ml) was added, and the solution heated at 100°C for five minutes. After cooling until just warm to the touch, the digested sample was carefully transferred through filter paper (5A, Toyo Roshi Kaisha Ltd, Japan) into a calibrated test tube. The volume was adjusted to 10 ml and split for each measurement.

SAMPLE ANALYSIS

All glassware was decontaminated by cleaning with a neutral detergent, rinsing with hot water to remove all detergent, washing with HNO_3 diluted 1:1 with water, and rinsing with deionised water at least five times.

A flame spectrophotometer ((FS), Hitachi 180-30 type (Japan)) was employed for measurement of Na and K. The operating conditions were: wavelengths of 589.0 nm for Na, 766.5 nm for K; slit setting 0.2 nm; flame air-acetylene. Gas chromatography for MMC measurement was performed with a Shimadzu GC-8A (Japan) gas chromatograph equipped with an electron capture detector (GC-ECD) and a glass column (3 mm $\times$ 1 m packed with Shimalite uniport (60-80 mesh) coated with DEGS-Hg). Column and injection/detector temperatures were 160°C and 230°C , respectively. Highly purified nitrogen was used as the carrier gas at a flow rate of 60 ml/min. Total mercury (T-Hg) analysis was carried out by cold vapour atomic absorption spectrometry (AAS).

Inductively coupled plasma atomic emission spectrometry (ICP-AES, Shimadzu ICPQ-1000, Japan, equipped with a professional computer) was used for the measurement of major, minor, and trace elements in rat organs. The operating conditions were power supply, 1.2 KW; coolant argon flow 11.3 l/min; carrier argon flow, 1.0 l/min; plasma argon flow, 1.4 l/min; nebulizer, concentric; plasma viewing height 15 mm; rate of introduction of sample, 2.3 ml/min (in demineralised water); plate current 0.75 A; integration time 20 seconds; analytical lines, Mg 279.55 nm, Ca 393.37 nm, Fe 259.94 nm, Mn 257.61 nm, Al 396.15 nm, Cu 324.75, Zn 202.55 nm.

The plasma was ignited according to manufacture's instructions, and the parameters set as previously outlined. The sample solutions were aspirated until the method file was completed. After measurement of every sample or standard, deionised

water was aspirated for about one minute to rinse the spray chamber and injector tube. For measurement of major elements the sample solution was diluted before analysis, and minor and trace elements were generally determined without dilution. To avoid effects of solids on the nebulizer (H-57 type) system and to minimise possible background and matrix interference owing to high concentration of acids, the sample was diluted with deionised water when required. Each element wavelength was calibrated using a single element standard. Calibration curves for each element were made by using four different standard solutions (including 0 $\mu\text{g}/\text{ml}$). Interference effects of the major elements Na, K, and Mg on the ICP-AES measurement were tested at four concentrations (0, 250, 500, and 1000 $\mu\text{g}/\text{ml}$) in solutions containing each of six minor and trace elements of 1 $\mu\text{g}/\text{ml}$ concentration.

DATA ANALYSIS

Differences in the time dependent changes of various essential elements in mercury exposed rat organs in comparison with controls were assessed for significance by analysis of covariance to test the difference on the population regression line of the element. Significance was evaluated at $p < 0.05$, $p < 0.025$, and $p < 0.01$.

From the principal component analysis (PCA), the factor contribution and factor score of each element were calculated on each exposed group by the correlation coefficient matrix including the time course data of all elements analysed in the three organs.

Results

Table 1 summarises the concentrations of various major, minor, and trace elements in the organs from HgCl_2 and MMC dosed rats and controls. These data were obtained from a time course study three, six, 12, and 24 hours after dosing. Total mercury concentrations were highest in the rat kidney after treatment with MMC and HgCl_2 , and these values were significantly greater compared with those in controls and also with concentrations in other organs. For the brain Na concentrations were lower in the HgCl_2 group than in the MMC and control groups, and K concentrations were highest in the MMC group. The brain concentrations of Ca and Mn in the HgCl_2 group and brain concentrations of Cu and Zn in the MMC group were also higher. For HgCl_2 exposed rat liver: higher Cu concentrations were found, compared with the MMC and control groups. For the kidney Cu concentrations were significantly higher in the HgCl_2 group and Zn concentrations were higher in the MMC group. Also, higher Na and lower K concentrations were found in the HgCl_2 group compared with the MMC and control groups. Figures 1 and 2 show the concentration changes with time of Ca

Table 1 Concentration changes of various major, minor, and trace elements in HgCl₂ and MMC exposed rat organs

Organ	Group	Na	K	Mg	Ca	Al	Fe	Mn	Cu	Zn	T-Hg
Brain	HgCl ₂	578 (130)* 393-846†	2410 (615) 1460-3460	147 (46.2) 79.2-225	140 (73.3) 56.2-310	0.97 (0.41) 0.45-1.82	24.9 (13.6) 7.69-61.4	0.27 (0.10) 0.11-0.40	1.02 (0.42) 0.68-2.08	11.2 (2.22) 7.31-15.0	0.049 (0.038) 0.003-0.127
	MMC	884 (500) 364-2000	3090 (1150) 1730-5100	144 (33.5) 86.4-216	105 (89.0) 19.1-345	0.75 (0.57) 0.21-2.20	13.6 (4.24) 9.09-24.5	0.18 (0.07) 0.08-0.31	1.54 (0.83) 0.80-3.33	22.6 (22.6) 9.09-80.6	0.95 (1.06) 0.004-3.75
	Control	813 (355) 500-1910	2580 (453) 1590-3270	126 (32.7) 66.7-178	92.8 (46.7) 40.9-193	0.66 (0.30) 0.31-1.25	18.0 (7.33) 6.33-31.8	0.14 (0.09) 0.02-0.30	1.01 (0.30) 0.62-1.64	10.1 (2.27) 7.14-13.6	0.020 (0.015) 0.003-0.055
Liver	HgCl ₂	1330 (364) 552-2210	1710 (310) 1170-2450	162 (41.4) 113-253	34.3 (14.9) 18.5-60.4	0.22 (0.08) 0.14-0.37	95.3 (29.3) 59.5-144	1.47 (0.28) 1.11-1.96	27.0 (31.9) 1.37-97.9	22.4 (5.66) 15.0-33.0	0.201 (0.130) 0.046-0.567
	MMC	1230 (494) 422-2020	1840 (533) 1040-2730	174 (14.2) 154-202	29.0 (4.87) 21.8-37.0	0.21 (0.03) 0.16-0.25	158 (39.4) 99.1-227	1.14 (0.20) 0.87-1.33	4.21 (2.37) 1.61-8.63	25.3 (4.40) 19.2-33.5	3.36 (2.12) 0.755-6.67
	Control	1580 (438) 804-2300	2170 (437) 1590-3170	171 (37.6) 133-256	27.8 (6.92) 19.5-40.9	0.21 (0.05) 0.13-0.30	165 (41.2) 115-228	1.24 (0.23) 0.91-1.73	4.78 (3.83) 1.41-12.1	22.2 (4.12) 16.9-29.9	0.013 (0.014) 0.001-0.050
Kidney	HgCl ₂	1080 (576) 214-2220	1180 (516) 222-1820	150 (46.6) 91.7-248	52.2 (23.3) 25.6-98.1	0.41 (0.14) 0.24-0.68	58.4 (18.4) 32.4-91.7	0.50 (0.18) 0.12-0.81	9.77 (10.2) 2.64-31.6	15.0 (3.53) 10.6-21.9	4.71 (3.64) 0.033-11.3
	MMC	918 (391) 500-2000	1520 (682) 650-2760	179 (22.7) 150-216	57.8 (19.4) 41.4-111	0.42 (0.13) 0.33-0.78	45.1 (26.7) 16.7-86.4	0.39 (0.11) 0.25-0.61	3.31 (1.39) 1.42-5.94	33.9 (33.9) 13.6-109	14.9 (17.4) 0.600-62.1
	Control	544 (461) 450-2100	1380 (1060) 333-4250	154 (40.4) 92.5-228	53.5 (15.9) 17.8-76.7	0.38 (0.12) 0.19-0.63	44.3 (13.1) 22.2-65.0	0.34 (0.15) 0.11-0.67	1.90 (0.64) 0.90-3.30	15.8 (5.26) 9.00-26.3	0.021 (0.007) 0.013-0.032

*Mean (standard deviation) µg/g wet tissue.

†Range.

Figure 1 Concentration changes of Ca and Zn in the mercury exposed rat brain. Three values for each element were obtained for each treatment and time.

Figure 2 Concentration changes of Cu and Zn in the mercury exposed rat kidney. Three values for each element were obtained for each treatment and time.

and Zn in rat brains and of Cu and Zn in rat kidneys from the two treated groups and controls.

Table 2 shows the results from the analyses of covariance on population regression lines of various elements in the three organs studied. Major elements, Na, K, and Ca, in organs exposed to a dominant lethal dose of HgCl₂, significantly increased in concentration (other than K in the liver). This was particularly so for all elements in the kidney. Some significant increases or decreases in concentrations of trace and minor elements such as Cu, Zn, Mn, and Fe were shown for both HgCl₂ and MMC groups. Also, the elements Mg, Ca, Al, and Cu in three organs exposed to HgCl₂, had comparatively good correlations with controls.

Discussion

Our present data (table 1) were comparable with reference data on concentration of essential elements in male Sprague Dawley rat tissues, bovine liver, etc.¹¹⁻¹⁸ Some essential elements in mercury exposed groups were shown to have significant changes in their concentrations in rat organs. For the rat kidney and brain rapid uptake of Zn within three hours was induced by exposure to MMC, and time dependence of increased concentrations of Cu was shown after HgCl₂ dosing. A significant increase in Cu concentration was also shown after exposure to MMC, although this was not time-dependent. To define the changes more clearly PCA was used. Figures 3 (HgCl₂ exposure) and 4 (MMC exposure)

Table 2 Results from analyses of covariance on population regression lines of various elements in rat organs between mercury exposed and control groups

Element	Brain		Liver		Kidney	
	HgCl ₂	MMC	HgCl ₂	MMC	HgCl ₂	MMC
Na	0.762*** 1.23	-0.519 -3.51	0.293*** 1.33	-0.155 -1.04	0.767*** 5.49	-0.562 -2.97
K	0.483*** 3.69	-0.639* -9.94	0.240*** -0.923	-0.409 -2.95	0.402*** 2.58	-0.762 -6.89
Mg	0.907* 0.524	0.692 0.314	0.901 0.463	0.513*** 0.099	0.866*** 0.505	0.383 0.118
Ca	0.777*** 0.784	0.280 0.338	0.916*** 0.169	0.933 0.062	0.962*** 0.281	0.650 0.171
Al	0.774*** 0.432	0.247 0.189	0.921*** 0.087	0.862 0.035	0.924*** 0.159	0.728 0.124
Fe	0.167 0.029	0.593 0.034	0.825 0.301	0.697 0.372	0.704*** 0.162	0.594** 0.215
Mn	0.661 0.001	0.568 0.001	0.753 0.003	-0.688*** -0.002	0.733*** 0.002	0.307 0.0004
Cu	0.861** 0.005	0.142 0.002	0.849*** 0.368	0.786* 0.025	0.854*** 0.109	-0.253 -0.005
Zn	0.617 0.017	-0.411* -0.126	0.706 0.050	-0.357*** -0.021	0.697*** 0.031	-0.536*** -0.246
T-Hg	0.115 0.537	-0.143 -23.4	0.069 1.11	-0.393 -110.0	0.877*** 400.0	-0.129 -350.0
MMC		0.861 0.194		-0.488 -0.822		-0.721 -4.38

*p < 0.05, **p < 0.025, ***p < 0.01.

The test of the difference for each element was conducted by using two population regression lines. The population of each element was constituted by all 12 values from four time points of three, six, 12, and 24 hours after dosing. The values show the correlation coefficients (above) between mercury exposed and control groups for essential elements and the regression coefficients (below) of mercury exposed groups.

Figure 3 Principal component analysis score projection on PC1-PC2 for essential elements in HgCl₂ exposed rat organs. Each of three elemental concentration data from a data point in the HgCl₂ exposed group was divided by a mean value of three control data and used as input data. The corrected data numbers for each element in each organ were 12. The PCA was performed using the correlation matrix.

Figure 4 Principal component analysis score projection on PC1-PC2 for essential elements in MMC exposed rat organs. Each of three elemental concentration data from a data point in the MMC exposed group was divided by a mean value of three control data and used as input data. The corrected data numbers for each element in each organ were 12. The PCA was performed using the correlation matrix.

show the PCA score projections, and suggest the time dependent characteristics of elements in rat organs induced by dosing with mercury. Figure 3 shows that several elements have abnormal scores on PC1-PC2 space, calculating the first factor contribution of 60% and the cumulative factor contributions of 88% up to the third factors. Concentrations of Cu in the liver and kidney and Mn in the brain may have abnormal characteristics; the uptakes of Cu and Mn by these organs were significant as shown in tables 1 and 2. Also, from a score projection on PC1-PC2 space for MMC exposure (fig 4) obtaining the cumulative factor contributions of 63% up to the third factors, Ca and Zn abnormalities with a wide variation appear to be considerable (see also table 1 and figure 1), although the abnormal scores of elements were not so clearly shown compared with some elements after exposure to HgCl_2 . A longer term study of rats exposed to MMC may more clearly define the apparently affected behaviours of Zn in the kidney, Cu in the brain and liver, and Mn in the brain.

Bogden *et al* have suggested that the increased concentrations of Cu in the kidney may be related to the induction of renal metallothionein synthesis by mercury¹¹; the addition of high concentrations of mercury to diets for rats causes the accumulation of mercury in renal metallothionein and causes Zn to accumulate in it.¹⁹ The concept that removal of metal is an important step in the degradation of metalloth-

ionein in vivo has also been discussed.²⁰⁻²² Funk *et al* have reported that the in vitro affinity of elements for metallothionein is $\text{Zn} < \text{Cd} < \text{Cu} < \text{Hg}$.²³ Mercury is thought to produce its renal toxicity by combining with plasma membrane sulphydryl groups in proximal tubular epithelium to form mercaptide bonds.²⁴ Furthermore, Chavez *et al* have reported that Hg^{2+} at concentrations higher than $5 \mu\text{M}$ induces Ca^{2+} efflux and the pathways of kidney mitochondrial Ca^{2+} release are associated with the binding of Hg^{2+} to membrane proteins.²⁵

From our statistical results the elements Na, K, Ca, Mn, Cu, and Zn showed significant concentration changes with time after a dominant lethal dosing with mercury compounds. Particularly, it was found by PCA that Cu in the liver and kidney and Mn in the brain after HgCl_2 exposure and Ca and Zn in the brain after MMC exposure exhibited specific behaviours at an early stage in three rat organs, compared with other essential elements as measured by simultaneous multielemental analysis.

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