Int Arch Occup Environ Health (1988) 61:65–69

International Archives of
Occupational and Environmental Health

Enzymuria in workers exposed to inorganic mercury

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Summary. Urinary excretion of beta-hexosaminidase (NAG = N-acetyl-beta-glucosaminidase) and albumin was examined in 41 chlor-alkali workers exposed to inorganic mercury and 41 age-matched controls. Either U-HG or B-Hg levels for these workers were available dating from the 1960s to the present. Increased U-NAG was seen in workers with a U-Hg today of more than 4 µg/mmol creat (about 50 µg/l; 35 ug/g creat). Multiple linear regression analysis showed that U-NAG was correlated to U-Hg and integrated dose but not to the present B-Hg level. No albuminuria (detection limit 12.5 mg/l) was found in any of the subjects. In a longitudinal study, no decrease in U-NAG levels was seen in 15 chlor-alkali workers after their vacation ($\bar{x}$ = 20 d). In five workers followed for ten months after a short exposure period, no definite time trend could be seen. The results show that there is a slight effect on renal tubules even at rather low levels of exposure to mercury vapour. The clinical significance of the enzymuria levels found here is, however, debatable.

Key words: Inorganic mercury – Occupational exposure – Biological monitoring – Kidney – Enzymuria

Introduction

It is well known that exposure to inorganic mercury may cause glomerular and tubular renal disease (Berlin 1986). Several cases of human glomerulonephritis induced by exposure to inorganic mercury have been described (Kazantzis et al. 1962; Tubbs et al. 1982). Some authors (Buchet et al. 1980; Roels et al. 1982) have found an increased prevalence of glomerular proteinuria in groups with occupational exposure to mercury, while others have found no such effects (Stonard et al. 1983). Mercuric chloride produces acute tubular necrosis after low doses, but metallic mercury is far less toxic to the proximal renal tubule. In the cross-sectional studies of workers occupationally exposed to metallic mercury vapour there was no low molecular weight tubular proteinuria (Buchet et al. 1980; Roels et al. 1982; Stonard et al. 1983).

However, renal dysfunction can also be detected in terms of increased activity of enzymes in urine. One such enzyme is beta-hexosaminidase (NAG = N-acetyl-beta-glucosaminidase), a hydrolytic enzyme located mainly in the lysosomes of the proximal tubular cells. Excretion of this enzyme is known to be increased in patients with impaired renal function in a variety of kidney diseases. Raised urinary NAG-levels have been reported in patients with glomerulonephritis, urinary tract infections, diabetes mellitus, hypertension and in patients treated with lithium drugs (Hultberg 1980; Thysell et al. 1982; Alderman et al. 1983; Hanseus et al. 1983; Sandberg et al. 1986).

Urinary NAG is considered a valuable tool in monitoring workers exposed to industrial toxins, and raised urinary levels of this enzyme could be the first sign of tubular dysfunction (Price 1982). This has previously been evaluated among groups occupationally exposed to mercury. In one study the mean U-NAG activity did not differ between chlor-alkali workers exposed to mercury vapour and non-exposed controls (Stonard et al. 1983). However, within the exposed group there was a statistically significant correlation found between urinary NAG and urinary mercury on the one hand and duration of exposure on the other. Subjects with Hg exceeding 100 µg/g creatinine

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had higher U-NAG activity than those with lower exposure. In another investigation 35 workers exposed to phenyl-mercuric acetate and mercuric chloride were studied (Meyer et al. 1984).

The urinary NAG activity was found to be elevated as compared with a control group. Blood mercury was determined in half of the exposed group. Within this group there was no correlation found between U-NAG and B-Hg, while a correlation was found between U-NAG and U-Hg levels. Subjects with U-Hg higher than 150 µg/l had significantly higher U-NAG than subjects with lower urinary mercury levels. No studies have been made concerning reversibility of enzymuria after discontinued exposure to mercury.

In chlor-alkali plants chlorine, hydrogene and sodium hydroxide are produced through electrolysis of a brine solution. Metallic mercury constitutes the continuously flowing cathode in the electrolysis cells.

The aim of this study was to find out whether kidney damage or dysfunction, as measured using U-NAG and U-albumin, could be shown in a group of chlor-alkali workers exposed to low levels of mercury vapour and, if it was shown, whether such effects were reversible.

Subjects

Part 1. Cross-sectional study

We examined 41 male workers with occupational exposure to inorganic mercury (mainly mercury vapour) at a chlor-alkali plant and 41 age-matched controls among blue and white-collar workers at the same company (office, transport, production outside the chlor-alkali plant). The mean age of exposed and controls was 36 years (SD = 12 years). The mean duration of exposure to inorganic mercury was 10 years (SD = 7 years). The controls had no actual or historical occupational exposure to mercury. None were exposed to other heavy metals, organic solvents or nephrotoxic chemicals. Subjects with known renal diseases or diabetes mellitus were not included in the study. One subject (exposed) had taken acetylsalicylic acid, but none used any more potent nephrotoxic drugs.

Part 2. Longitudinal study

With the aim of studying changes in urinary NAG activity after discontinued exposure to mercury, 15 workers were examined before and after a vacation period of 17 to 26 d (mean = 20 d). These workers had been exposed to mercury for 1 to 20 years (mean = 9 years). We also measured U-NAG repeatedly in five other workers after short exposure (3 d) to high levels of mercury vapour (no previous exposure).

Methods

In the cross-sectional study (Part 1) the 41 exposed subjects and the 41 controls were examined simultaneously in pairs over a three-week period. Blood and morning urine samples were collected in metal-free tubes. Plasma was separated and all samples were frozen the same day and stored at −25°C. Samples from the five workers in the longitudinal study were handled in the same fashion. For practical reasons urinary samples from the 15 workers in Part 2 had to be stored in +6°C for 1 to 2 months before being frozen.

Mercury in whole blood, plasma and urine was analysed using the cold vapour atomic absorption technique. Each sample was analysed in duplicate. Accuracy was tested and found acceptable in comparison with other laboratories using cold vapour AAS and neutron activation analyses, respectively. The precision, as calculated from duplicate analyses, was satisfactory. In the relevant range of U-Hg-concentrations (10–200 µg/l) the coefficients of variation (CV) were 7 and 4.9% for the low and high values respectively. The CV of P-Hg-values was 4.3% and there was no significant difference between high and low values (range 3–25 µg/l). Assay of NAG was performed colorimetrically using p-nitrophenyl-2-acetoamido-2-deoxy-beta-D-glucopyranoside (Koch-Light, Colnbrokk, England) as the substrate (Hultberg and Wieslander 1982). The coefficient of variation was less than 5%. Urinary albumin was measured by electro-immuno-assay, using human protein (Kabi-Vitrum, Stockholm) as the standard. The detection limit was 12.5 mg/l.

In addition to B-Hg, P-Hg and U-Hg, a cumulative exposure dose was calculated for each subject from previous B-Hg and U-Hg levels, which had been determined since the start of biological monitoring in 1961. The dose was calculated by integrating B-Hg as a function of exposure time. In the 1960s only U-Hg was determined. Therefore, for this study. U-Hg was then transformed into B-Hg by dividing by a factor of 2.5 (Skerfving et al. 1984). Three men exposed to mercury had been working at the chlor-alkali plant before 1961. Their exposure during the 1950s had been estimated by an occupational hygienist. Smoking habits, fish meals per week and recent dental care were also registered.

NAG activity and urinary Hg were corrected for urine flow rate, by expressing the results in terms of creatinine.

When comparing the groups statistically, Student's *t*-test for paired observations was used. For correlation between U-NAG and various measures of exposure, linear and multiple linear regression analyses were used.

Results

Mercury exposure and urinary NAG in the cross-sectional study of 41 exposed subjects and 41 controls are shown in Table 1. Today's levels of B-Hg and U-Hg are low, roughly corresponding to an air level of 25 µg/m^3 (Roels et al. 1987). A comparison of all exposed subjects and all controls reveals no difference between the groups. There is, however, a statistically significant difference between the exposed subgroup with a present level of U-Hg > 4 µg/mmol creat (U-Hg > 35 µg/g creat corresponding to about U-Hg > 50 µg/l) and the controls. Exposed subjects with B-Hg > 10 µg/l or an integrated dose of B-Hg > 160 µg × years/l were also found to have statistically significant elevations of U-NAG as compared to the controls. Within the exposed group there is a slight positive correlation between U-NAG and U-Hg, P-Hg,

Table 1. Urinary NAG activity, urinary mercury (U-Hg), blood mercury (B-Hg) and accumulated dose in workers exposed to mercury and unexposed controls

Group	*n*	U-NAG U/mmol creat mean (SD)	U-Hg[a] (mean)		B-Hg µg/l mean	Dose[b] µg years/l mean
			µg/mmol creat	µg/g creat		
Exposed	41	0.17 (0.13)	3.4[c]	30[c]	9.2[d]	160[e]
U-Hg > 4 µg/mmol creat	13	0.22 (0.14)*	6.0	53	14.4	176
B-Hg > 10 µg/l	13	0.23 (0.13)**	5.1	45	15.6	198
Dose (B-Hg) > 160 µg years/l	15	0.23 (0.14)*	3.2	28	10.2	322
Controls	41	0.15 (0.12)	0.4	3.7	3.4	–

* $P < 0.05$ (one-tailed)
** $P < 0.01$ (one-tailed)
[a] Morning samples: mean U-creat 1.5 g/l
[b] B-Hg levels for each individual integrated over time
[c] Range 0.9–10.7 µg/mmol creat; 8–94 µg/g creat
[d] Range 1.6–32.0
[e] Range 3–861

Table 2. Multiple linear regression analysis with U-NAG as the dependent variable. All R^2-values differ significantly from zero

Independent variables	R^2
U-Hg	0.13*
P-Hg	0.12*
B-Hg	0.10*
Dose	0.09*
U-Hg, U-creat	0.29*
U-Hg, dose	0.24*
P-Hg, dose	0.21*
B-Hg, dose	0.19*
U-Hg, P-Hg	0.15
U-Hg, B-Hg	0.15
U-Hg, U-creat, dose	0.41*
U-Hg, U-creat, P-Hg	0.32
U-Hg, dose, P-Hg	0.25
U-Hg, dose, B-Hg	0.25
U-Hg, U-creat, dose, P-Hg	0.44

* All parameters contribute significantly to the model ($P \leq 0.05$)

B-Hg and integrated dose, respectively (Figs. 1, 2 and Table 2). The exposure variables are, however, intercorrelated, so the data were analysed using multiple linear regression. Present U-Hg (corrected for creatinine) is the strongest single predictor for U-NAG. Adding integrated dose increases the fraction of the variance explained, while adding B-Hg or P-Hg does not. Adding U-creatinine to the equation makes the data fit the model even better.

Not a single case of albuminuria was detected. All exposed subjects and controls had urinary albumine concentrations lower than the detection limit (12.5 mg/l).

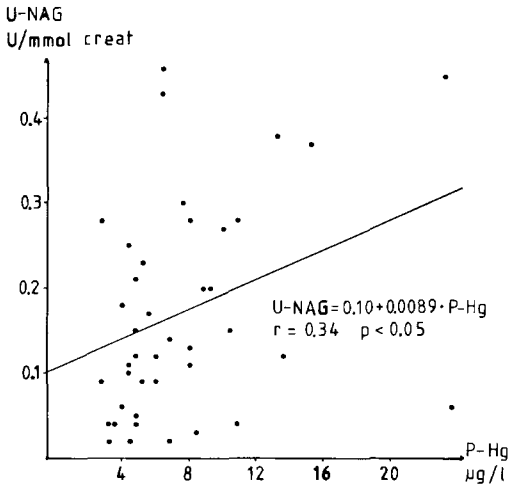

Fig. 1. Correlation between urinary NAG activity (U-NAG) and plasma mercury (P-Hg) in 41 chlor-alkali workers

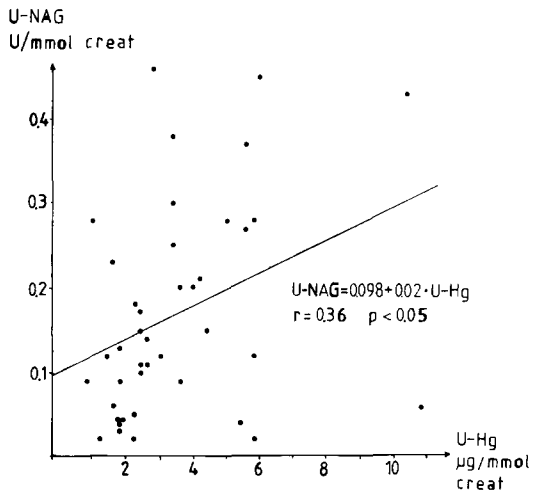

Fig. 2. Correlation between urinary NAG activity (U-NAG) and urinary mercury (U-Hg) in 41 chlor-alkali workers

Table 3. Urinary NAG activity and mercury levels in 15 chlor-alkali workers after cessation of exposure to mercury

Time	U-NAG U/mmol creat mean (SD)	U-Hg µg/l mean	U-Hg/creat µg/mmol mean	U-Hg/creat µg/g mean	P-Hg µg/l mean
Before vacation	0.26 (0.14)	51	3.5	31	15
After vacation	0.25 (0.15)	60	3.3	29	6.5

Table 4. Urinary NAG activity and urinary mercury in 5 workers examined after 3 d of high exposure to mercury.
I = Immediately after exposure ceased (mean of 2 d).
II = After four months (mean of 2 d).
III = After ten months (mean of 3 d)

Subject no.	U-Hg (µg/mmol creat)			U-NAG (µ/mmol creat)		
	I	II	III	I	II	III
1	3.5	1.5	0.8	0.14	0.11	0.09
2	2.3	0.8	0.3	0.07	0.10	0.05
3	2.0	1.2	0.7	0.19	0.10	0.12
4	1.2	0.7	0.7	0.06	0.09	0.06
5	1.5	0.5	0.1	0.08	0.04	0.06
Mean	2.1[a]	0.9[b]	0.5[c]	0.11	0.09	0.08

[a] 18 µg/g creat
[b] 8 µg/g creat
[c] 4 µg/g creat

Exposure and urinary NAG values in the longitudinal study can be seen in Tables 3 and 4. In 15 workers urinary NAG was found not to change over a mean vacation period of 20 d. In the five workers examined over a longer time period after exposure ceased, NAG-levels after four and ten months tended to be somewhat lower, but the time trend was not statistically significant.

Discussion

Our study showed slightly elevated urinary NAG activity but no albuminuria among workers exposed to mercury, as compared with an unexposed control group. The enzymuria is probably caused by an effect on the renal proximal tubules (Price 1982). Increased excretion of U-NAG among workers exposed to inorganic mercury was also found in another study (Stonard et al. 1983), but only among subjects with U-Hg > 100 µg/g creatinine. In our study, the effect on urinary NAG activity was seen in workers with lower present levels of exposure to mercury (U-Hg > 4 µg/mmol creatinine; U-Hg > 35 µg/g creat; U-Hg > 50 µg/l). Our material was analysed statistically in a somewhat different way than the above-mentioned study where prevalence of U-NAG "above upper limits" was used. Our results are more in agreement with those in a Belgian study (Roels et al. 1985) where the urinary activity of another enzyme, beta-galactosidase, was increased when U-Hg exceeded 50 µg/g creatinine.

When using urinary NAG activity as an indicator of renal damage the values should be corrected for creatinine to eliminate dilution effects (Price 1982). The same holds true for urinary mercury (Barber et al. 1986). In very much diluted or concentrated urinary samples, the creatinine concentration is, however, no longer inversely proportional to the urine flow rate (Price 1982). A complicating factor is that high urinary creatinine concentrations may, in part, be caused by large muscle mass. The muscle mass is, on the other hand, positively correlated to renal mass, which tends to increase urinary NAG activity (Price 1979). Correcting urinary NAG activity and urinary mercury for dilution effects by dividing with creatinine concentration is, therefore, not an ideal procedure, and this may explain why introducing creatinine as another factor in the regression equation results in better predictions.

For urinary NAG, the scatter from the mean is considerable, even in the controls. As can be seen in Table 1 the coefficient of variation is about 80% (0.12/0.15). In our own experience, about half of this variation is intra-individual and could be reduced by using the mean value of urinary NAG activity from several days. This may be important when looking for slight tubular damage in small groups of workers exposed to nephrotoxic agents.

In the 15 workers examined before and after an exposure-free vacation period of about 20 d, urinary NAG did not change. This is not surprising, as urinary mercury excretion was about the same before and after the vacation, indicating a biological half time for U-Hg on the order of months rather than weeks (Piotrowski et al. 1975; Cherian et al. 1978). Blood mercury, however, declined to less than half the initial values. In the five workers who were examined over a longer period of time, there was, similarly, no statistically significant change in urinary NAG activity. This may be explained by the fact that the kidney was not affected in those subjects, or by the fact that the number of subjects was too small to indicate the small decrease in NAG levels. Therefore, our study gives no answer to the question of whether or not the elevated urinary NAG activity among workers exposed to mercury could be reversible after a long (more than 20 d) exposure-free period.

The enzymuria could have been the result of leakage, caused by an effect of mercury exposure on tubular cell membranes. The contribution of cells in urine is probably small (Price 1982). The clinical significance of enzymuria among subjects exposed to inorganic mercury is unclear. Urinary NAG activity is a very sensitive indicator for renal dysfunction. All our exposed subjects were clinically healthy when the study was undertaken. The elevation of urinary NAG is of the same order as has been found among subjects taking lithium drugs or subjects treated for hypertension (Thysell et al. 1982; Alderman et al. 1983). The levels are lower than can be seen among subjects with juvenile diabetes mellitus (Hanseus et al. 1983). A transient elevation of U-NAG can also be seen among subjects with urinary tract infections, with no subsequent renal disease.

Although the clinical significance of enzymuria among workers exposed to mercury is questionable it seems reasonable to strive for exposure levels that do not affect the kidney. Our study indicates that this would mean urinary mercury (in morning samples) below 4 μg/mmol creatinine (35 μg/g creat; 50 μg/l), corresponding roughly to an air level of 25 $\mu g/m^3$ (WHO 1980; Roels et al. 1987). This level was also suggested by the WHO Study Group as a TLV for occupational exposure (WHO 1980).

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Received September 28, 1987 / Accepted June 23, 1988