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Elevation of Urinary N-Acetyl-β-D-glucosaminidase and β-Galactosidase Activities in Workers with Long–Term Exposure to Aromatic Nitro-Amino Compounds

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Aromatic nitro-amino compounds are used as raw materials of dyes, rubber, pesticides and drugs. A common toxicological reaction to these compounds is the formation of methemoglobin followed by cyanosis and anemia (Beard and Noe 1981). An epidemiological study has indicated that mortality from heart disease is elevated in workers exposed to an aromatic nitro-amino compound (Levine et al. 1986).

Some analgestics or antifebrics containing aromatic nitro-amino compounds have nephrotoxicity as a side effect (Stygles and Iuliucci 1981). Little is known about renal damage in workers occupationally exposed to aromatic nitro-amino compounds. Recently, certain urinary enzyme activities have been measured as an index of renal damage (Piperno 1981). In animal experiments (Ito et al. 1987; Yoshida and Hara 1988), the activity of urinary N-acetyl-β-D-glucosaminidase (NAG) was increased in rats given p-aminophenol. Here, we assayed urinary enzyme activities of workers handling aromatic nitro-amino compounds, and discuss the nephrotoxic effects of these compounds used industrially.

MATERIALS AND METHODS

Sixty-two male Japanese workers in a chemical factory aged 20 to 64 years (mean ± SD, 41.8 ± 12.2) were our subjects. They worked the day shift (8 a.m. to 4 p.m.) in a 5-day workweek. In this factory, the workers routinely handle aromatic nitro-amino compounds, including p-aminophenol, o-aminophenol, p-nitrophenol, p-anisidine, p-nitroaniline, p-nitrochlorobenzene, o-nitrochlorobenzene, p-phenetidin, ethylaniline and 4-chloro-2-aminophenol as raw materials for the production of dyes or drugs. For the past two decades, the yearly amounts of aromatic nitro-amino compounds handled have been almost constant. The workers were divided into two groups; one (n=35) was of workers who routinely handled the aromatic nitro-amino compounds and the other (n=27) was of office workers, who served as the controls. Each group was further

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divided into 3 sub-groups according to age. Table 1 lists the numbers in each sub-group and give the duration of exposure.

Table 1. Number of subjects and duration of exposure at work

Age	Number of subjects		Duration of exposure (yr)*
	Office workers	Exposed workers	
20-34	6	11	3.6 ± 2.5
35-49	14	10	12.9 ± 6.7
50-64	7	14	18.1 ± 4.3

*, Values are means ± SD.

The main duties of the exposed workers were the putting of the aromatic nitro-amino compounds from paper bags into reaction vessels, the taking out the reaction products from the reaction vessels, and the bagging of the reaction products into paper bags. These jobs were done at separate rooms in the chemical factories. A few exposed workers did the carrying of the paper bags containing the aromatic nitro-amino compounds or the reaction products by folk lift trucks and the warehousing of them. The workers wore fabric protective clothing and cotton gloves on their duties. Only when the workers were engaged in the bagging, they wore particulate-filter respirators and poly-vinyl chloride coated cotton gloves.

A questionnaire information obtained on each subject regarding smoking and drinking habits indicated that the consumption of alcoholic drinks and tobacco was similar in all groups. Also, serum biochemical examination performed at an another day showed no difference in serum aspartate aminotransferase (GOT), serum alanine aminotransferase (GPT), serum γ-glutamyltranspeptidase (γ-GTP), serum alkaline phosphatase (ALP) and serum urea nitrogen (BUN) among all groups.

Urine samples were collected from the subjects at 10 a.m. to 12 noon on a Thursday in June. Urine samples were also collected in the morning after a five-day summer vacation in August.

Urinary diazo-positive metabolites were assayed by the method of Watanabe et al. (1976) with p-aminophenol as the standard material. All the subjects were carefully questioned to find if they had taken any cold remedy, analgesic, anti-allergy drug, or antihypertensive drug, and data from those with affirmative answers were eliminated. Urinary creatinine was estimated with a commercial kit (Creatinine-Test, Wako Pure Chemical Industries, Ltd., Osaka).

Within 24 h of the urine sampling, the activities of urinary NAG, γ-GTP, β-galactosidase (β-GAL) and ALP were assayed. The β-GAL was assayed by the method of Kresse et al. (1982). The assay medium was composed of 50 μL of the urine sample and 200 μL of 0.1 M sodium citrate buffer (pH 4.0) containing 0.1 M NaCl, 6 mM NaN_3, 0.2% bovine serum albumin, and 10 mM p-nitrophenyl-β-D-galactopyranoside. After incubation at 37°C for 30 min, 2 mL of 0.4 M glycine-NaOH buffer (pH 10.4) was added to the assay medium, and the p-nitrophenol released was measured with a spectrophotometer at the absorbance of 410 nm. The three other urinary enzyme activities were assayed by the use of commercial kits; for NAG, the NAG-Test SHIONOGI (Shionogi Pharmaceuticals Ltd., Osaka); for γ-GTP, the γ-GTP C-Test (Wako); and for ALP, the Alkaline Phospha B-Test (Wako).

Experimental data were treated statistically by two-way analysis of variance (Two-way ANOVA) (Snedecor and Cochran 1967). The results for the urinary diazo-positive metabolites and the urinary enzyme activities were logarithmic-normal distributions, so data from them were logarithmically transformed before the statistical treatment.

RESULTS AND DISCUSSION

Table 2. Urinary diazo-positive metabolite levels in workers

Age	Diazo metabolites (mg/mg creatinine)*	
	Office workers	Exposed workers
20-34	0.36 (0.28 - 0.48)	0.50 (0.34 - 0.70)
35-49	0.38 (0.22 - 0.61)	0.54 (0.38 - 0.76)
50-64	0.31 (0.22 - 0.44)	0.50 (0.38 - 0.69)

*, Values (geometrical means with SD ranges in parentheses) are expressed as the p-aminophenol equivalent. Results of statistical treatment by two-way ANOVA were as follows: effect of exposure, $p < 0.01$; effect of age, not significant (NS); effect of "exposure x age", NS.

The excretion of urinary diazo-positive metabolites in June is shown in Table 2. Although urine was not sampled at the end of the shift, the levels of diazo-positive metabolites were significantly higher ($p < 0.01$) in the exposed workers than the office workers. There was no variation with age.

The environmental concentrations of the aromatic-nitro amino compounds were not measured here, but we could draw some conclusion about the probable exposure levels of the subjects as follows. In a study of workers exposed to about 0.38 mg/m^3 of p-nitrochlorobenzene (Yoshida et al. 1988), the results showed 0.54 mg/mg creatinine as the level of urinary diazo-positive metabolites on a Thursday morning in July. In this study, in which sampling was done on a Thursday morning in June, the exposed workers excreted diazo metabolites at the level of about 0.5 mg/mg creatinine on the average. Thus, these subjects were probably exposed to aromatic nitro-amino compounds at more than 0.3 mg/m^3 as p-nitrochlorobenzene. In addition, because the amounts of these compounds handled yearly were almost constant over the last two decades, the workers seemed to have been exposed routinely to this level of nitro-amino compounds throughout their employment.

Aromatic nitro-amino compounds readily penetrate the intact skin (Beard and Noe 1981). In the factory presently surveyed, the aromatic nitro-amino compounds directly contacted with the workers' skin on their duties. Additionally, respirators were wore by the workers only when they did the bagging. So, it is likely that the aromatic nitro-amino compounds were absorbed both via penetration through intact skin and via respiratory tract.

In Table 3, activities of the four urinary enzymes are given. With NAG and β-GAL activities, effects on enzyme levels of the exposure to the aromatic nitro-amino compounds were significant ($p< 0.05$); NAG and β-GAL activities were increased in the exposed workers 50 years of age or older. Activities of urinary γ-GTP and ALP did not vary with age or exposure to the aromatic nitro-amino compounds.

In the factory surveyed, the workers had a 5-day summer vacation. Table 4 shows the diazo metabolite levels and NAG activities in urine collected from the workers just after the vacation. The diazo metabolite levels of the exposed workers had decreased to those of the office workers; however, the NAG activities of the exposed workers aged 50 or more remained high. These results indicate that the NAG activity was chronically elevated in the older exposed workers.

Table 3. Activities of urinary enzymes in workers*

Age	NAG		γ-GTP		β-GAL		ALP	
	Office workers	Exposed workers	Office workers	Exposed workers	Office workers	Exposed workers	Office workers	Exposed workers
20-34	1.5 (1.1-1.9)	1.2 (0.8-1.8)	13.2 (9.8-17.8)	11.1 (8.7-14.2)	4.0 (3.0-5.4)	3.9 (3.0-5.0)	4.2 (2.8-6.3)	4.0 (2.9-5.5)
35-49	1.5 (0.8-2.9)	2.1 (1.2-3.7)	12.2 (7.8-19.2)	13.8 (8.4-22.5)	3.9 (2.5-6.2)	5.7 (4.1-7.9)	4.4 (2.5-7.6)	4.2 (2.8-6.1)
50-64	1.8 (1.1-2.9)	4.1 (1.9-8.7)	12.7 (7.4-21.9)	11.7 (7.3-19.0)	4.8 (3.4-6.9)	6.3 (4.3-9.3)	4.5 (2.9-6.9)	5.3 (3.3-8.5)
Two-way ANOVA								
Exposure	$p < 0.05$		NS		$p < 0.05$		NS	
Age	$p < 0.01$		NS		$p < 0.05$		NS	
Exposure x Age	$p < 0.05$		NS		NS		NS	

*, Values (geometrical means with SD ranges in parentheses) are expressed as units per gram of creatinine.

Table 4. Urinary diazo-positive metabolite levels and NAG activities in workers after a 5-day holiday*

Age	Diazo metabolites**		NAG (unit/g creatinine)	
	Office workers	Exposed workers	Office workers	Exposed workers
20-34	0.26 (0.19-0.37)	0.35 (0.26-0.47)	1.7 (1.4-2.1)	1.4 (1.0-2.1)
35-49	0.28 (0.18-0.44)	0.29 (0.19-0.43)	1.9 (1.0-3.5)	3.0 (1.7-5.4)
50-64	0.30 (0.21-0.43)	0.39 (0.30-0.51)	1.9 (1.2-2.9)	3.9 (2,0-7.3)
Two-way ANOVA				
Exposure	NS		$p < 0.05$	
Age	NS		$p < 0.05$	
Exposure x age	NS		NS	

*, Values are geometrical means with SD ranges in parentheses.
**, Expressed as milligram of p-aminophenol equivalent per milligram of creatinine.

Among the aromatic nitro-amino compounds handled in the factory surveyed, p-aminophenol and p-chloroaniline have toxic effects on rat kidneys (Crowe et al. 1979; Rankin et al 1986). p-Nitrophenol, p-anisidine and p-nitrochlorobenzene also handled by the exposed workers may be metabolized to these nephrotoxic aromatic nitro-amino compounds in vivo. In this study, the exposed workers aged 35 or more have handled nitro-amino compounds, including such nephrotoxic substances, for more than 10 years. Thus, it is likely that the long-term exposure to these nitro-amino compounds has caused chronic renal damage in the exposed workers and resulted in the elevation of urinary NAG and β-GAL.

The elevation of urinary NAG induced by a single dose of nephrotoxic substance disappears within 56 hr (Lockwood and Bosmann 1979). On the other hand, the continued elevation of urinary NAG excretion following repeated administration of nephrotoxic anti-inflammatory analgesics was reported (Burry et al. 1976). Here, NAG was elevated in the urine collected the morning after a 5-day period without exposure to the nitro-amino compounds; NAG activity was chronically elevated. Thus, urinary NAG excretion can be utilized to monitor not only acute but also chronic renal damage.

Urinary γ-GTP and ALP activities did not vary with exposure or age. γ-GTP and ALP localize in the plasma membrane of tubular epithelial cells in the kidney, and NAG and β-GAL are abundant in the lysosomes. The lysosomal enzymes may be more sensitive indicators of renal tubular damage than the membrane enzymes.

Urinary NAG is elevated in workers handling mercury (Himeno et al. 1986) and in residents of an area polluted with cadmium (Sugihira and Saito 1986). Urinary lysosomal enzymes are useful in screening for renal damage in industrial workers, just as serum enzymes are useful in screening for liver damage.

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