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Professional Paper

BILIARY FUNCTION IN WORKERS OCCUPATIONALLY EXPOSED TO ALUMINIUM DUST AND FUMES*

Milka BOGDANOVIĆ and Petar BULAT

Institute of Occupational Health "Dr Dragomir Karajović", Belgrade, Serbia

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This study investigated billiary secretory function in workers occupationally exposed to aluminium dust and fumes. It included a group of 34 male workers aged (44.1±7.8) years and exposed up to 4.6 mg m^{-3} of aluminium dust and fumes in workplace air for (21.6±2.5) years, and a group of 30 unexposed control male workers. Serum and urine aluminium levels were measured in both groups before and after chelating treatment with 1 g deferoxamine by intramuscular injection. Billiary function was assessed by measuring gamma-glutamyl transpeptidase, alkaline phosphatase, 5-nucleotidase, cholesterol and its fractions, total and indirect bilirubin, and bile acids. We then analysed the relationship between Al exposure and billiary function.

In the exposed group mean serum aluminium was significantly higher [(4.91±3.86) μg L^{-1}] than in controls. The same was true for urine Al before [(1.57±1.93) μg L-1] and after deferoxamine [(11.51±14.97) μg L^{-1}]. Total and indirect bilirubin and alkaline phosphatase were significantly higher in the exposed than in control workers, and they correlated with urine Al after the chelating treatment.

Our findings suggest that chronic occupational exposure to aluminium dust and fumes leads to a significant body retention of aluminium. The impaired biliary secretion in the exposed workers manifested itself in subclinical signs of cholestasis.

KEY WORDS: *aluminium, hepatic secretory function, occupational exposure*

Aluminium (Al) is ubiquitous in human environment. For many years people had thought that Al was not toxic, but when a connection between Al and neurological disturbances in patients on dialysis was found, more research of Al toxic effects followed. It has been discovered that large amounts of Al can cause neurological, bone and lung disorders, anaemia, glucose intolerance, and cardiac arrest. Exposure to Al may also cause irritation of the eyes and respiratory tract (1-7).

Al hepatotoxic effects are not considered important, even though Al partially aggregates in hepatocyte lysosomes (5). However, recent experimental studies have shown that Al can cause cholestasis. Al has been associated with a disbalance in the transport of organic molecules through sinusoidal and canalicular membrane, which is then reflected in the disturbance of bilirubin and bile salts emission (4, 6).

In view of this effect on the billiary secretory function, the aim of our study was to assess signs of cholestasis in workers exposed to Al dust and fumes.

SUBJECTS AND METHODS

We examined a group of 34 workers who had been occupationally exposed to high Al concentrations in the form of dust and fumes for many years (the exposed group). They worked in Al electrolysis, in

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potrooms, in anode assemblies, electrolytic baths, and in the processing of liquid aluminium. The exposed workers were male, (44.1±7.8) years of age. Their average working experience was (21.6±2.5) years, while the exposure time was (12.7±4.1) years. The average Al concentration in the working atmosphere was 4.6 mg m^{-3}.

The control group consisted of 30 men who had never been occupationally exposed to Al (controls). Their average age was (43.1±6.4) years.

We recorded subjective complaints and objective findings, laboratory blood and urine tests, and serum and urine Al before and after the implementation of a chelating agent. The same was performed in 30 control subjects.

Al concentrations in serum and urine were determined using a "Unicam" SP 90 A electrothermal atomic absorption spectrometer (ET AAS) (7). Reference values for serum Al were (1.6 to 7.5) μg L^{-1} and for urine Al from (13±6) μg L-1 to (61±22) μg L^{-1} per day.

Having obtained a written informed consent from the subject, we treated them with deferoxamine, a chelating agent, in order to estimate Al body burden. Deferoxamine is commonly indicated for treatment of exposed workers, if serum Al concentration exceeds 10 μg L^{-1}, (8, 9). Deferoxamine was applied intramuscularly, in the dose of 1 g a day for three consecutive days (10).

To evaluate billiary secretory function, we used the following parameters: gamma-glutamyl transpeptidase (GGT) according to the Szasz method, alkaline phosphatase (ALP) according to the Bassey method, 5-nucleotidase (5-NU) according to the Rosalka-Wilson method, total and indirect bilirubin according to the Jendrassik-Groff method and glicocholic and glicochenodeoxicholic bile acids (CH and CHDK) according to the radioimmunoassay (RIA) method on a gamma scintillation counter "Compo Gamma 1282" by Hofman (7).

The obtained data were processed for descriptive statistics, Student's t-test, Mann-Whitney U-test, and multiple correlations test using the SPSS program (Statistical Package for the Social Sciences).

RESULTS

Table 1 shows serum and urine Al concentrations (before and after the application of the chelating agent). In the exposed workers they were significantly higher ($p<0.01$) than in controls. After provocation the difference was even more pronounced ($p<0.005$).

Liver elimination function is shown on Table 2. Total and direct bilirubin were higher in the exposed workers, while conjugated bilirubin did not differ between the groups. Blood bile acid concentration

Table 1 *Aluminium concentrations in serum and urine*

Subjects	Aluminium / μg L^{-1}		
	Serum	Urine 1	Urine 2
Exposed	4.91±3.86	1.57±1.93	11.5±14.97
Control	2.15±2.77	0.26±0.29	1.23±1.48
Level of significance*	p<0.01	p<0.01	p<0.005

Results are expressed as arithmetic means and standard deviations
Urine 1: urine Al before administration of 1 g deferoxamine im;
Urine 2: urine Al during three days of administration 1 g deferoxamine im.
**(Student's t-test)*

Table 2 *Bilirubin and bile acid blood concentrations as biomarkers ofliver elimination function*

Subjects	Bilirubin / mmol L^{-1}			Bile acids / μmol L^{-1}	
	Total	Direct	Indirect	CH	CHDK
Exposed	14.2±3.58	4.54±3.17	10.1±3.37	1.41±1.05	2.36±2.07
Control	12.3±3.7	2.36±0.75	10.0±3.17	1.3±1.29	2.01±2.61
Level of significance*	p<0.04	p<0.001	Not significant	Not significant	Not significant

Results are expressed as arithmetic means and standard deviations
CH - glicocholic; CHDK - glicochenodeoxicholic
**(Student's t-test)*

Table 3 *Alkaline phosphatase, gamma-glutamyl–transpeptidase, and 5-nuleosidase as biomarkers of cholestasis*

Subjects	ALP / U L^{-1}	5-NU / U L^{-1}	GGT / U L^{-1}
Exposed	30.6±8.01	1.85±0.99	7.55±4.68
Control	23.7±9.62	1.58±1.02	7.7±4.82
Level of significance*	p<0.003	Not significant	Not significant

Results are expressed by arithmetic means and standard deviations
ALP - alkaline phosphatase; GGT – gamma-glutamyl-transpeptidase; 5-NU - 5-nucleotidase
**(Student's t-test)*

was higher in the exposed workers than in controls, but the difference was not statistically significant.

Table 3 shows the results of the test for cholestasis. Alkaline phosphatase activity was significantly greater in the exposed workers. Glicocholic and glicochenodeoxicholic bile acids were within normal limits and did not show statistical deviation between the groups.

Using correlation tests we established positive correlations between total bilirubin and total extracted Al content and also between indirect bilirubine and blood Al (Table 4).

Table 4 *Multiple correlation test results*

	Al-U1	Al-serum	Al-U2
Total bilirubin	0.209	0.175	0.506*
Direct bilirubin	0.068	0.644*	0.091
Indirect bilirubin	0.139	0.357	0.395
CH	0.376	-0.401	0.39
CHDC	0.273	-0.357	0.294
ALP	0.062	0.4	0.246
5-NU	0.256	0.202	0.141
GGT	-0.31	0.323	0.037

Al-U1: urine Al before administration of 1 g deferoxamine im.
Al-U2: urine Al during the three days of administration of 1 g deferoxamine im.
CH-glicocholic, CHDC-glicochenodeoxicholic, ALP-alkaline phosphatase, 5-NU- 5 nucleotides, GGT-gamma-glutamyl-transpeptidase.
**(significant correlation)*

DISCUSSION

In normal healthy adults serum and urine Al is about (4 to 10) μg L-1 and (3 to 30) μg L-1, respectively. Urine Al in exposed workers can reach up to (100 to 300) μg L-1 (2, 5, 6), depending on exposure intensity and duration.

The half-life of Al in human body after acute exposure is eight hours. During chronic, long-lasting exposure, Al accumulates in various tissues, bones, spleen, heart, and liver. Overall Al body load in non-exposed persons reaches (35 to 40) mg (2, 5, 6). In exposed persons this body load is several times as high, and can be estimated by giving a chelating agent such as deferoxamine (8-10).

The exposed workers in our study have clearly shown increased Al body content, because serum and urine Al, before and after the administration of the chelating agent, were significantly higher than in non-exposed subjects. A statistically significant increase in both total and indirect bilirubin and alkaline phosphatase indicate cholestasis.

Our findings are in agreement with the results of other experimental studies of Al effects on billiary secretory function. In rats sub-chronically poisoned with Al bile salts increased in the while bile production dropped (11). Changes were also observed in bile acids glycine and taurine (12).

The latest research by Gonzales et al. (4) has shown that Al poisoning results in cholestasis, as it disturbs the transport of organic ions through sinusoidal and ductular membranes. Having in mind that the transport of organic ions is in close correlation with liver elimination of endo- and exotoxins, including bilirubin, bile salts, leukotriene C4, drugs and other substances, its disturbance can significantly affect the liver function.

However, the mechanism through which Al produces this effect is not even close to being explained. Gregus et al. in 1980 (13) and Ming-Tsang et al. in 1997 (14) found that Al disturbed protein synthesis, resulting in lower concentrations of a huge number of enzymes which take part in phase I and II detoxification such as gluthatione-S-transferase, catalase, and glutathione peroxidase.

Latest research shows a very important role of oxidative stress. Al increases lipid peroxidation and at the same time decreases antioxidative activity of enzymes such as gluthatione-S-transferase, catalase and glutathione peroxidase (15, 16).

Oxidative stress caused by Al poisoning leads to a reduction of transportation molecules in the ductular membrane, which could be the cause for disturbed billiary secretory function (4).

CONCLUSION

Our workers with long-term exposure to Al dust and fumes showed a significantly increased Al body burden. In these workers the parameters of cholestasis, total and indirect bilirubin, and alkaline phosphatasewere significantly higher than in unexposed persons, even when they kept within reference limits. These findings show that prolonged exposure to Al induces cholestasis and impairs billiary secretory function in occupationally exposed workers, and call for inclusion of alkaline phosphatase in periodical checkups of Al-exposed workers.

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Sažetak

BILIJARNA FUNKCIJA U RADNIKA PROFESIONALNO IZLOŽENIH ALUMINIJSKOJ PRAŠINI I DIMU

Eksperimentalna istraživanja na životinjama pokazuju da kronična izloženost aluminiju može izazvati smanjen prijenos organskih aniona preko žučnih kanalića, što ima za posljedicu poremećaje sekrecije žuči i kolestazu. Učinci kronične izloženosti aluminiju na bilijarnu funkciju u ljudi do sada nisu istraživani. Procjenjivali smo učinke na bilijarnu funkciju radnika koji su profesionalno izloženi prašini i dimu aluminija. U izloženoj skupini bila su 34 muškarca, životne dobi (44,1±7,8) godina koji su tijekom (21,6±2,5) godina bili izloženi razini do 4,6 mg m^{-3} prašine i dima aluminija. Kontrolna skupina sastojala se od 30 neizloženih radnika. Vrijednosti aluminija određene su u serumu i mokraći u obje skupine prije i nakon davanja kelatirajućeg spoja (deferoksamin u dozi od 1 g im.). Za procjenu bilijarne funkcije rabljeni su ovi pokazatelji: γ-glutamil transpeptidaza, alkalna fosfataza, 5-nukleozidaza, kolesterol, ukupni i indirektni bilirubin te žučne kiseline. Analizirana je korelacija između izloženosti aluminiju i bilijarne funkcije.
Srednja vrijednost Al u serumu izloženih radnika [(4,91±3,86) μg L^{-1}], kao i koncentracije Al u mokraći prije [(1,57±1,93) μg L^{-1}] i nakon primjene kelatirajućeg spoja [(11,5±15,0) μg L^{-1}] bile su statistički značajno više u odnosu na vrijednosti u kontrolnih ispitanika. Vrijednosti ukupnog i indirektnoog bilirubina te alkalne fosfataze bile su statistički značajno više u izloženih radnika i pozitivno su korelirale s ukupnim Al izlučenim mokraćom nakon primjene kelatora. Može se zaključiti da kronična profesionalna izloženost prašini i dimu aluminija dovodi do tjelesnog opterećenja aluminijem i poremećaja bilijarne funkcije, što se odražava supkliničkim znakovima kolestaze.

KLJUČNE RIJEČI: *bilijarna sekrecija, aluminijum, profesonalna ekspozicija*

CORRESPONDING AUTHOR:

Milka Bogdanović, MD, PhD
Institute of Occupational Health "Dr Dragomir Karajović"
Deligradska 29, 11000 Beograd, Serbia
E-mail: *mlkbogdanovic@yahoo.com*