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Research Paper

Effects of gallium, indium, and arsenic dose biomarkers and malondialdehyde on zinc protoporphyrin and DNA fragments in optoelectronic workers

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Abstract

Purpose: To investigate whether occupational exposure to gallium, indium, and arsenic may disrupt normal heme production and damage DNA by initiating the release of lipid peroxidation.
Methods: 101exposed optoelectronic workers and 65 referents were tested for their whole blood and urine levels of gallium, indium, and arsenic. Plasma malondialdehyde (MDA), blood zinc protophorphyrin (ZPP), and serum DNA fragments were used as indicators of toxic hazards.
Results: The MDA, ZPP, and DNA fragment mean levels in the exposed workers were significantly higher than those in the referents. The levels of urine gallium in the exposed workers were significantly correlated with the levels of whole blood ZPP and serum DNA fragments respectively. MDA (the production of lipid peroxidation) may be one of the mechanisms for gallium-related DNA damage in multiple regression models. In addition, gallium was found to have a synergistic effect with arsenic on MDA level that may induce indium to damage DNA in blood cells.
Conclusion: ZPP and DNA fragments can be elevated by gallium exposure, and the MDA increase due to combined exposure to gallium and arsenic may play a role in indium-induced DNA damage.

Keywords: *Optoelectronic; ZPP; DNA Fragments.*

1. Introduction

Optoelectronic manufacturing is a metallic-intensive modern industry. The optoelectronic industry has been a rapidly expanding industrial sector in many countries. Toxic metals of the periodic table group ⅢA and ⅤA are widely used in the semiconductor and optoelectronic industries (Robinson 1983, Edelman 1990, Fowler et al. 1993). Occupational exposure to gallium (Ga), indium (In), and arsenic (As) habe been described as significantly higher in these exposed groups than in the referents (Liao et al. 2004). These metals or metalloids are known to alter several cellular functions, initiate apoptosis, and cause carcinogenic effects in mammals (Fowler et al. 1993. Woods et al. 1979, Aoki et al. 1990, Chang et al. 2003, Zhou et al. 2005). Liao et al found correlations between the levels of urine Ga and as, and the plasma levels of MDA in optoelectronic workers (Liao et al. 2006).
ZPP, which indicates a disruption of normal heme production, is widely accepted as a general mechanism for Pb poising, iron deficiency, chronic inflammatory disorder, and porphyrias (Chisolm et al. 1975, Rosel et al. 1990, Hastka et al. 1993, Harthoorn-Lasthuizen & Sant 2000). Rossi et al showed that human lymphocyte ferrochelatase was inhibited by in vitro Cu and Hg (Rossi et al. 1990). In addition, Labbe noted that a variety of metals (Pb, Cd, and Hg) that react with sulfhydryl (-SH) groups are capable of inhibiting purified ferrochelatase (Labbe et al. 1987). DNA fragments indicate damage to DNA before cell death, which also acts as an initial hallmark of apoptosis (Distelharst 1988, Konishi et al. 2003). This oxidative damage may play an important role in many pathological conditions like cancer, autoimmunity conditions, and Alzheimer's disease (Oehm et al. 1992, Carson & Ribeiro 1993, Hegde et al. 2004).
Optoelectronic manufacturing is a modern metallic-intensive industry. In the field of occupational and environmental health, the effects of Ga, in, and as on human erythropoiesis and DNA synthesis have not been fully investigated. ZPP is a specific element to evaluate if employees are at increased risk for material impairment to their heme synthesis (Distelharst 1988). DNA fragmentation is a sensitive and specific aspect to evaluate whether employees are at risk of oligonucleosome impairment to the appropriate regulation of cell functions (Bonfoco et al. 1995, Uwai et al. 2000, Mishima et al. 2002). In this study, the blood MDA, ZPP and DNA fragment levels of optoelectronic workers were determined in an attempt to be established whether occupational exposure to Ga, in and as may disrupt a normal heme production and DNA synthesis by initiating the release of lipid peroxidation.

2. Methods

2.1. Subjects

The study population included 103 optoelectronic workers and a reference group of 67 controls from industrial located in the north of Taiwan. There were no workers with iron-deficiency anemia, or with anemia of chronic disorder. Four workers were excluded

because of having past history of thyroid, and tumor disease. Therefore, the final study population consisted of 101 optoelectronic workers (15 fabrication equipment preventative workers, 51 dopants and thin film workers, and 36 fabrication supervisors and engineers). The general characteristics of the exposed workers and referents are shown in Table1. The study was approved by the Institutional Review Board in Kaohsiung Medical University. All participants were informed and signed consent forms.

2.2. Collection and analysis of biomonitoring specimens

Personal first spot of urine specimen in the morning and blood sample from the antecubital vein were obtained from the study subjects during their health check- ups. Subjects were instructed to eat nothing for at least 8 hours prior to blood and urine sampling. Blood and urine specimens were kept at -20oC prior to analysis (Pan 1993). The levels of heavy metals were determined by inductively coupled plasma-mass spectrometry after microwave dissolution. The methods for metals and MDA analysis have been previously presented (Liao ET al.2004, Liao et al. 2006). The detection limits (3SD of 10 unexposed urban residents) of blood Ga, in, and as were 0.016 μg/L, 0.081μg/L, and 0.970μg/L. The detection limits of urine Ga, in, and as were 0.101 μg/L, 0.005μg/L, and 9.980μg/L. The measurements below a concentration of detection limits were set at half of the detection. MDA was determined by high-performance liquid chromatography. The coefficient of variation for the interassay of precision was 3.83 % the coefficient of variation for the intraassay of precision was 5.08 %.

2.3. Determination of whole blood ZPP and serum DNA fragments

Blood (whole blood in EDTA) was collected, and 1 ml whole blood was transported. Care was taken not to exposure to light contamination. The blood was stored at 2-8oC
For no longer than five wk prior to analysis. ZPP levels in blood were measured with a hematofluorometer (Aviv Lakewood, New Jersey, USA). There were no hemolyzed, or clotted samples. Serum was taken from the supermatants of centrifuged blood. The serum was stored at 2-8 oC for no longer than five wk prior to analysis. Histone- associated-DNA-Fragments level (mU = absorbance [10-3] was measured using cell death detection ELISA kit (Roche, Cat. No. 11774425001, Germany). The 20μl of serum was transferred to a 96-well micro-titer plate. After adding 80μl of immune reagent to each well, samples were incubated on a MP shaker for two hours at 15-25 oC. Wells were washed, and each well pipette with 100μl of ABT substrate solution. Absorbance was measured at 450 nm (reference wavelength: 690nm)(Hingst & Blottner 1995).

2.4. Statistics

All results were presented as the value of means, or median. When two groups were compared. The Student's test or Chi-square test were employed. The limit of significance was set at $p < 0.05$. The correlation in the various groups was studied by Pearson's regression after the data had been normalized. The data were also subjected to multiple linear regression models using the SPSS program (SPSS Inc, Chicago, IL) for Windows.

3. Results

Table1 shows the demographic data by exposure situation. There was a higher proportion of males, smoking habit, and consuming vitamin complex tablets regularly in the exposed group than in referents.

Table 1: Subjects Characteristics of Exposed Workers (N = 101) and Referents (N = 65).

Items	Exposure group	Referents
Median		
Duration of employment (months)	22.80	24.00
Age (yrs)	28.20	29.07
Body mass index (kg/m^2)	22.32	23.00
Sex*		
male	62	22
female	39	43
Education level		
< college	80	51
≧college	21	14
Smoking status*		
no	81	61
yes	20	4
Alcohol consumption		
no	88	59
yes	13	6
Vitamin complex intake*		
no	75	58
yes	26	7

*X^2 test: exposure group versus referents, df = 1, $p < 0.05$.

3.1. Exposure and health outcome measures

The levels of metals, MDA, ZPP, and DNA fragments in subjects are shown in table2. The mean blood in (0.22 ± 0.17 ppb), urine Ga (0.24 ± 0.22 ppb), and as (32.69 ± 27.06 ppb), plasma MDA (0.98 ± 0.44μmol/L), blood ZPP (36.46 ± 5.81μmol/mol heme), and serum DNA fragments levels (240.37 ± 41.78 mU) were significantly higher in the occupationally exposed workers than in the referents.

Table 2: Metal Levels (Ppb), MDA (Mmol/L), ZPP (Mmol /Mol Heme) and DNA Fragmentation (Mu) in Blood and Urine among the Exposed Workers (N = 101) and Referents (N = 65).

Body fluid	Parameter examined	Exposure group	Referents
Blood (median)	Ga	0.51 ± 0.34 (0.47)	0.48 ± 0.23 (0.46)
	In*	0.22 ± 0.17 (0.17)	0.14 ± 0.12 (0.11)
	As	8.70 ± 6.36 (7.25)	7. 89 ± 6.60 (6.78)
	Pb	34.49 ± 11.29 (34.78)	32.05 ± 12.55 (31.49)
Urine	Ga*	0.24 ± 0.22 (0.19)	0.15 ± 0.09 (0.15)
	In	0.03 ± 0.04 (0.01)	0.02 ± 0.03 (0.01)
	As*	32.69 ± 27.06 (25.49)	24.65 ± 22.68 (22.66)
	Pb	29.72 ± 16.54 (25.90)	26.64 ± 19.57 (22.50)
Plasma	MDA*	0.98 ± 0.44 (0.96)	0.67 ± 0.33 (0.67)
Blood	ZPP*	36.46 ± 5.81 (35.58)	29.32 ± 4.62 (28.75)
Serum histone-associated -DNA-fragments*		240.37 ± 41.78 (239.09)	209.72 ± 46.11 (210.72)

*: Student's t test, significant difference, $p < 0.05$ (exposed workers vs. referents, df = 1, all data had been normalized).

3.2. Associations between metals and health outcome measures

Among the 101 optoelectronic workers, urine Ga levels were positively and significantly correlated ($p < 0.05$) with plasma MDA, blood ZPP, and serum DNA fragment levels respectively ($r = 0.35$, 0.47, and 0.39). Blood In levels were correlated ($r = 0.26$, $p < 0.05$) with serum DNA fragments. Urine As levels was correlated ($r = 0.22$, $p< 0.05$) with plasma MDA levels. As indicators of

health outcomes in exposed workers, plasma MDA levels were positively and significantly correlated ($r = 0.43$, $p < 0.05$) with serum DNA fragments, but were not significantly correlated with blood ZPP levels in the exposed group. There were no any significant correlations between metals in blood or in urine and plasma MDA, blood ZPP, and serum DNA fragments in the referents (Table3).

Table 3: Pearson's Correlation Coefficient between Metal Levels in Blood, Urine, Plasma, MDA, Blood ZPP, and Serum DNA Fragments (DNAF) Levels.

Parameter	Exposure group	Referents	Total
Blood			
In vs MDA	0.09	0.07	0.15
In vs ZPP	0.19	0.09	0.18
In vs DNAF	0.26*	-0.04	0.22*
Urine			
Ga vs MDA	0.35*	0.30*	0.37*
Ga vs ZPP	0.47*	-0.02	0.32*
Ga vs DNAF	0.39*	0.26	0.39*
As vs MDA	0.22*	-0.09	0.17
As vs ZPP	0.14	0.06	0.13
As vs DNAF	0.09	-0.04	0.09
Blood			
MDA vs ZPP	0.14	0.14	0.16
MDA vs DNAF	0.43*	0.05	0.30*

*: significant difference, $p < 0.05$, (all data had been normalized).

3.3. Effects of MDA adjustments on Ga level associations with ZPP and DNA fragments measure models

Associations between the urine Ga and the health outcomes, after adjustment for MDA were modeled in the exposed workers because the association of Ga biomarker with MDA and the associations between higher Ga dose and ZPP levels and DNA fragments were also found in the exposed group. As shown in table 4, association between the Ga measures and DNA fragments remained significant after adjustment for MDA. The regression coefficient (β) of urine, Ga was 0.328 ($p < 0.05$). Other risk factors such as MDA ($\beta = 0.314$, $p < 0.05$), cigarette smoking ($\beta = 0.301$, $p < 0.05$), and alcohol consumption ($\beta = -0.221$, $p < 0.05$) also significantly affected the serum DNA fragmentation. Association between the Ga measures and ZPP levels remained constant after adjustment for MDA also. Other risk factors such as blood Pb levels, age, duration of employment, body mass index, sex, education levels, cigarette smoking, and alcohol consumption, when analyzed all the regression coefficients (β) did not significantly affect the blood ZPP levels.

Table 4: Linear Regression Models to Evaluate Association of Urine Ga and Plasma MDA Dose with Blood ZPP and Serum DNA Fragments Levels in 101 Optoelectronic Workers.

Model	Model1 (Ga)	Model2 (MDA)	Model 3 (combine)
Variables	β p value	β p value	β p value
DNA fragments models	($R^2 = 0.20$, $p < 0.05$)	($R^2 = 0.13$, $p < 0.05$)	($R^2 = 0.28$, $p < 0.05$)
Urine Ga	0.447 p < 0.05		0.328 p < 0.05
MDA		0.346 p < 0.05	0.314 p < 0.05
Smoking status no vs yes	0.260 p < 0.05		0.301 p < 0.05
Alcohol consumption no vs yes		-0.242 p < 0.05	-0.221 p < 0.05
ZPP models	($R^2 = 0.22$, $p < 0.05$)	($R^2 = 0.07$, $p > 0.05$)	($R^2 = 0.21$, $p < 0.05$)
Urine Ga	0.472 p < 0.05		0.464 p < 0.05
MDA			
Pb			

P value < 0.05, significant difference. All models were also adjusted for age, sex, BMI. Educational level and consuming vitamin complex tablets.

3.4. Associatios of blood in levels with DNA fragmentation by MDA levels above and below the median respectively

Different associations of blood in levels with DNA fragments were evident when workers with plasma MDA levels above and less than or equal 0.96μmol/L were compared (Table 5). The regression coefficients (β) of blood in, duration of employment, cigarette smoking, and alcohol consumption was 0.284, 0.318, and -0.287 respectively (all $p < 0.05$) in serum DNA fragments for workers with plasma MDA levels above 0.96μmol/L. The association of blood in levels with DNA fragments was more pronounced at MDA levels above 0.96μmol/L.

Table 5: Linear Regression Models of DNA Fragments on Blood in Levels By MDA Level above and Below 0.96 Mmol/L (Median) in 101 Optoelectronic Workers.

Variables	MDA > median β p value	MDA > median β p value
	($R^2 = 0.25$, $p < 0.05$)	($R^2 = 0.16$, $p > 0.05$)
Number	50	51
Blood In	0.284 p< 0.05	0.268 p > 0.05
Duration of employment		
Smoking status no vs yes	0.318 p < 0.05	
Alcohol consumption no vs yes	-0.287 p < 0.05	

P value < 0.05, significant difference. All models were also adjusted for age, sex, BMI. Educational level and consuming vitamin complex tablets.

3.5. Synergistic effect of exposure to Ga and As on MDA measures

Table 6 summarized the characteristics of study subjects stratified by median levels of urine Ga (0.19 ppb) and as (25.49 ppb) in a different exposure group. The median plasma MDA level was significantly higher in the H-Ha and H-As group than in the L-Ga and H-As group, as well as in the L-Ga and L-As group respectively. The median BMI was significantly higher in the H-Ga and L-As group than in the L-Ga and H-As group, as well as in the L-Ga and L-As group respectively. All other characteristics such as duration of employment, alcohol consumption, cigarette smoking, sex, age, and education level were not statistically significant between these four exposure groups respectively. Thus, there is a synergistic effect of Ga and as exposure on MDA level.

Table 6: Characteristics of Study Sybjects (N = 101) Stratified by Median Levels of Urine Ga (0.19 Ppb) and as (25.49ppb) in the Optoelectronic Industry.

Exposure	H-Ga & H-As	H-Ga & L-As	L-Ga & H-As	L-Ga & L-As
Number	30	22	22	27
MDA*	1.18	1.03	0.90[a]	0.80[a]
BMI *	23.2	24.7	21.7[b]	21.7[b]

H: median level, L: < median level. * kruska-Wallis H test, $p < 0.05$ (all categories of exposed groups, df = 3), [a] Mann-Whitney test, $p < 0.05$ (H-Ga & H-As vs L-Ga & H-As, and L-Ga & L-As respectively, df = 1). [b] Mann-Whitney test, $p < 0.05$ (H-Ga & L-As vs L-Ga & H-As, and L-Ga & L-As respectively, df = 1).

4. Discussion

This study showed an elevated blood In, urine Ga, urine. As, plasma MDA, blood ZPP, and serum DNA fragment levels in workers exposed to Ga, In, and As when compared with the referents. Association between increased MDA and higher Ga and as measures were found. In addition, serum DNA fragments were significantly correlated with urine Ga and blood. In levels. Blood ZPP levels were correlated with urine Ga levels only. These results suggest that heavy exposure to Ga may possibly disrupt a function of heme synthesis and a structure of DNA in human blood cells.
Urine Ga affecting DNA fragments was observed in participants in the exposed workers after adjustment for duration of employment,

alcohol consumption, smoking status, sex, age, education level, BMI, and consuming vitamin complex tablets. The association was diminished after adjustment for plasma MDA, although urine Ga remained significantly associated with DNA fragments in the 101 workers. The data indicated an effect of Ga on DNA fragmentation beyond that due to Ga exposure alone, and because MDA was associated with adverse DNA fragmentation and reduced the significance of the Ga biomarker association with DNA fragments in the exposed workers. Therefore, MDA may be one but not the only one mechanism by which Ga is cytopathogenetic to DNA fragmentation. MDA was not associated with adverse heme synthesis, and did not result in reducing the significance of Ga biomarker association with ZPP in the exposed workers. These results imply that the mechanism of Ga-induced dose-dependent ZPP and DNA fragments are different.

Ga resembles iron with respect to transferring-gallium (Tf-Ga) complexes result in iron deprivation that inhibits cellular proliferation and hemoglobin production (Chitambar & Zorica 1987, Willianm et al. 1996). The antiproliferative activity of Tf-Ga appears to be the result of a blockade of ribonucleotide reductase. This occurs both directly, by interfering with iron incorporation into the iron-containing M2 subunit of the enzyme (Chitambar et al. 1991, Narasimhan et al. 1992), and indirectly, by depriving the cell of intracellular iron necessary for ribonucleotide reductase activity (Chitambar et al. 1988). The enhancement of lipid peroxidation may help metal cause promutagenic damage that includes DNA base modifications, inter- and intramolecular crosslinking of DNA and proteins, DNA strand breaks, rearrangements, and depurination (Kasparzake 1995). Recent studies have shown that cellular uptake of Ga leads to activation of caspases and induction of apoptosis (Chitambar 2004). The Tf-Ga can also inhibit the hemoglobin synthetic pathway by decreasing the iron incorporation into heme, which indirectly accumulates zinc protoporphyrin in blood cells (Chitambar & Zorica 1987, Labbe et al. 1987).

Levels of MDA in the exposed workers were correlated significantly with the levels of urine Ga and as. However, urine Ga was the only significant risk factor, accounting for 33.9 % of the affects on plasma MDA level. Table 5 shows that exposure to Ga had a synergistic effect with As on lipid peroxidation of blood in the exposed workers. The results imply that combined exposure to Ga and As shows a dose-response relationship in association with MDA levels, although as is not the major risk factor.

The correlation between blood In levels and serum DNA fragments levels was modified by MDA levels in the optoelectronic workers. This sign was most pronounced at plasma MDA levels > median (0.96μmol/L). We showed that Ga and As induced lipid peroxidation activity in cell membrane, and thus hypothesize that MDA is a risk factor that induces indium penetration and damage to the inner nucleus by forming larger lipidic pores in the cell membrane.

In regression analysis, urine Ga level was the only factor affecting blood ZPP. Socioeconomic status and blood Pb did not affect blood ZPP levels significantly. Many studies have suggested that iron deficiency, chronic inflammatory, and Pb exposure could disrupt heme synthesis (Hastka et al. 1993, Alexander et al. 1998, Roh et al. 2000). The level of ZPP in erythrocytes may be 5- to 10-fold greater than normal levels in anemia of iron-deficiency or chronic disorders. It may be 10- to 250- fold greater than normal levels of ZPP level in clinically manifest Pb poising and in protoporphyria (Dagg et al. 1966, Granick et al. 1972, Hastka et al. 1993). We found that none of the workers had iron-deficiency anemia, or anemia of chronic disorder. We also found that the mean level of ZPP in exposed workers was about 1.2- fold (36.46/29.32 = 1.2) greater than the referents. We also found that the blood Pb mean levels in exposed workers (median = 34.78 ppb) were not significantly higher than blood Pb mean level in the referents (median = 31.49 ppb). According to these results, ZPP, as an index of disruption of heme synthesis may be ruled out by Pb exposure in this study.

The BMI did not affect the plasma MDA levels, although there were different BMI in exposed subgroups. Therefore, as a confounding factor BMI could be ruled out in the study of synergistic effect of exposure to Ga and As analyses. The highly reactive chemicals such as hydrogen fluoride, and fluoride compounds might induce the lipid peroxidation, DNA damage and apoptosis in animal cells (Wang et al. 2004, He & Chen 2006). These chemicals were most used in the etching operation room. In this study, theexposed workers who were employed in the operation of dopant and epitaxy. Theprocess usually use a lot of metals of a periodic table group ⅢA and ⅤA in the thin film room. They often submitted the processes of photolithography and etching to other contract companies. Therefore, the possible effects of the strong acid and solvent on lipid peroxidation, ZPP, and DNA fragmentation will be estimated in the future.

5. Conclusion

According to these findings, occupational exposure to Ga could increase the blood ZPP and DNA fragment's levels in optoelectronic industry workers. MDA may be one, but not the only, mechanism for Ga- related DNA damage. Together with exposure to As, Ga has a synergistic effect on MDA formation, which may change the cell membrane permeability and then induce indium to damage DNA in the cellular nucleus.

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