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Analysis of Time Dependent Low Level Exposure to Gallium Arsenide on Blood ALAD activity, Glutathione and Lipid per oxidation levels in Rat Blood, Liver and Kidney

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ABSTRACT

Gallium arsenide (GaAs), an intermetallic semiconductor has widespread applications in the electronic industry. GaAs has the ability to dissociate into its constitutive moieties, arsenic and gallium which might be responsible for the oxidative stress. The present study was aimed at evaluating, effect of gallium arsenide on blood ALAD activity, glutathione and lipid per oxidation levels in rat blood, liver and kidney on exposure of 1, 2 and 6 months. Result indicated that arsenic moiety in GaAs was mainly responsible for causing oxidative stress via increased TBARS levels, decreased glutathione levels in blood and tissues. We also noted the decreased activity of ALAD in rat blood on exposure to gallium arsenide. The study demonstrates that the time dependent exposure to low level gallium arsenide led to increased in lipid per oxidation, decrease glutathione level and ALAD activity which concludes that the slow release of arsenic moiety from GaAs is mainly responsible for oxidative stress in rats and exerts its toxicity in time dependent manner related to its dissolution and maximum toxicity by increasing the time period of exposure.

INTRODUCTION

Gallium arsenide is an intermettalic semiconductor consists of a metalloid (arsenic) and trivalent transition element (gallium), belonging to group IIIa-Va of the periodic table. It is extensively used for light-emitting diodes and semiconductor lasers for the optical data storage and playback system and the high-speed optical communication system due to its superior property of photon emitter. Gallium arsenide has distinct advantage in electronic speeds devices as compared to those of the semiconductor silicon; therefore it is increasingly used for the satellite communication system, the ultrafast supercomputer in the electronic industry. It is also used as popular semiconductor material for the solar cells. Gallium arsenide (GaAs) is one of the important components which possess superior properties for making frequency devices and photon emitters (Robinson, 1983). All these extensive uses of GaAs will inevitably lead to an increase in the exposure of workers manufacturing these products.

Exposure to gallium arsenide occurs during the sawing and polishing of GaAs wafers, industrial workers may inhale GaAs particles (Yamamuchi et al., 1989). Acute risk of occupational exposure to GaAs is not entirely clear, because studies have more focused on monitoring exposure levels (Flora, 1999) rather than accessing the chemicals impact on the worker's health, including their immuno-competency. Recent literature indicated that toxicity of GaAs is mainly regulated on the basis of inorganic arsenic toxicity although, gallium moiety might also be playing a crucial role in their toxicity (Webb et al., 1986). Chemical form of gallium arsenide is not as toxic as their dissolution products. Dissociation of gallium arsenide into its constituent moieties arsenic and gallium following oral and intratracheal instillation (Webb et al., 1987; Flora et al., 1994). It is suggested that gallium is the primary inhibitor of ALAD following dissolution of GaAs in vivo which either compete for or displaces zinc from the enzyme active site responsible for inhibition (Malachowski,1990). Arsenic, on the other hand, impairs cellular respiration by inhibiting mitochondrial enzymes and uncoupling oxidative phosphorylation via inhibition of sulfhydryl group containing cellular enzymes and substitution of phosphate with arsenate in “high energy” compounds (Mahaffey, 1995). These arsenate compounds are unstable and are rapidly hydrolyzed, termed as arsenolysis.

MATERIALS AND METHODS

Chemicals

Gallium arsenide was purchased from Aldrich chemicals, US (purity 99.99%). All other analytical laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or BDH chemicals (Mumbai, India). Ultra pure water prepared by Millipore (New Delhi, India) and was used throughout the experiment to avoid metal contamination and for the preparation of reagents and buffers used for various biochemical assays in our study.

Animals and Treatments

All animals were procured from animal house facility of Defence Research and Development Establishment (DRDE), Gwalior. All experiment were performed on male albino rats weighing 120±10 g housed in stainless steel cages in an air-conditioned room with temperature maintained at 25±2oC and 12 hours alternating day and night cycles. Rats were allowed standard chow diet (Amrut Feeds, Pranav Agro, New Delhi) throughout the study. Rats were allotted to two groups of 15 rats each and were treated as below for 1, 2 or 6 month-

Group I - Normal animals
Group II - GaAs, 0.5 mg/kg, oral (freshly prepared as suspension in 0.25 M phosphate buffer)

After 1, 2 or 6 months GaAs exposure was stopped while zinc and iron supplementation was continued. Five animals from each group were sacrificed after month 1, 2, and 6 under light ether anesthesia. Blood was collected by cardiac puncture in heparinized vials. Liver and kidney were rinsed in cold saline, blotted, weighted and used for various biochemical variables.

Experimental Protocol

Blood δ-aminolevulinic Acid Dehydratase (ALAD)

The activity of blood ALAD was assayed according to the procedure of (Berlin and Schaller, 1974). 0.2 ml of heparinized blood was mixed with 1.3 ml of distilled water and incubated for 10 min at 37oC for complete hemolysis. After adding 1 ml of standard **δ** -ALA, the tubes were incubated for 60 min at 37 oC. The reaction was stopped after 1 h by adding 1 ml of 10 % trichloroacetic acid (TCA). After centrifugation (1500 g) of reaction mixture equal volume of Ehrlich reagent was added to the supernatant and the absorbance was recorded at 555 nm after 5 min.

Blood glutathione (GSH)

Analysis of blood GSH concentration was performed with method described by (Ellman, 1959), and modified by (Jollow et al., 1974). In brief, 0.2 ml of whole blood was added to 1.8 ml of distilled water and incubated for 10 minutes at 37 oC for complete hemolysis. After hemolysis 3 ml of 4 % sulphosalycylic acid was added and tubes were centrifuged at 2500 rpm for 15 min. Supernatant (0.2 ml) was mixed with 0.4 ml of 10 mM 5, 5'dithiobis-(2-nitrobenzoic acid) (DTNB) and 1 ml phosphate buffer (0.1M, pH 7.4). At the end absorbance at 412 nm was recorded.

Liver and kidney reduced glutathione (GSH)

Liver and kidneys GSH levels were measured as described by (Hissin and Hilf, 1973). Briefly, 0.25 g of tissue sample was homogenized on ice with 3.75 ml of phosphate - EDTA buffer and 1 ml of 25 % HPO3 which was used as a protein precipitant. The total homogenate was centrifuged at 100,000 g for 30 min at 4oC. For the tissue GSH assay, 0.5 ml supernatant and 4.5 ml phosphate buffer (pH 8.0) were mixed. The final assay mixture

(2.0 ml) contained 100µl supernatant, 1.8 ml phosphates - EDTA buffer and 100µl o-phthaldehyde (OPT; 1000 µg/ml in absolute methanol, prepared fresh). After mixing, fluorescence was determined at 420 nm with an excitation wavelength of 350 nm using a spectrofluorometer (Model RF 5000 Shimadzu, Japan).

Thiobarbituric acid reactive substances (TBARS)

Tissue lipid peroxidation was measured by method of (Ohkawa et al., 1979) Tissue homogenate was incubated with 8.1 % sodium dodecyl sulfate (SDS) (w/v) for 10 minutes followed by the addition of 20 % acetic acid (pH 3.5). Reaction mixture was incubated with 0.6 % TBA (w/v) for 1 h in boiling water bath. Pink color chromogen formed was extracted in butanol-pyridine solution (15:1) and read at 532 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56 x105 /M/ cm.

Statistical Analysis

The results are expressed as the mean ± SEM of number of observations. Comparisons between means were carried out using Student's t test. Differences were considered significant at $P < 0.05$ unless otherwise stated in the text.

RESULTS

Effect of low level gallium arsenide exposure on Blood ALAD activity and glutathione level in rats

Effect of gallium arsenide on blood ALAD activity and glutathione level is shown in Table 1. Blood ALAD activity and glutathione level decreased on gallium arsenide exposure compared to normal animals. The effects become more pronounced in animals after increasing time period of exposure to gallium arsenide.

Effect of low level gallium arsenide exposure on few biochemical variables in rat liver

Effect of gallium arsenide on liver TBARS and glutathione levels is shown in Table 2 and 3. Liver TBARS level increased significantly in gallium arsenide exposed animals after exposure of 1, 2 and 6 months where as glutathione level decreased on exposure to gallium arsenide.

Table 1: Effect of low level of gallium arsenide exposure on few biochemical variables in rat blood month 1, 2 and 6.

Group	Liver			Kidney		
	GSH (mg/gm tissue)			GSH (mg/gm tissue)		
	Month 1	2	6	Month 1	2	6
Normal animal	5.56±0.17			2.63±0.20*		
GaAs	6.05±.0..85	5.00±0.46	4.15±0.34*	2.351±0.44	2.29±0.56*	2.07±0.13*

Values are meanSE; n=5. *P< 0.05 compared to normal animals as evaluated by Student's t test.

Table 2: Effect of low level of gallium arsenide exposure on few biochemical variables in rat liver on month 1, 2 and 6.

Group	Liver			Kidney		
	GSH (mg/gm tissue)			GSH (mg/gm tissue)		
	Month 1	2	6	Month 1	2	6
Normal animal	5.56±0.17			2.63±0.20*		
GaAs	6.05±.0..85	5.00±0.46	4.15±0.34*	2.351±0.44	2.29±0.56*	2.07±0.13*

Values are meanSE; n=5. *P< 0.05 compared to normal animals as evaluated by Student's t test.

Table 3: Effect of low level of gallium arsenide exposure on few biochemical variables in rat liver on month 1, 2 and 6.

Group	Liver			Kidney		
	TBARS (µg/g of tissue)			TBARS (µg/g of tissue)		
	Month 1	2	6	Month 1	2	6
Normal	1.88±0.19			5.35±0.76		
GaAs	2.11±0.71	2.39±0.16*	3.06±0.21*	5.56±0.98	5.67±0.54	8.85±0.21*

Values are meanSE; n=5. *P< 0.05 compared to normal animals as evaluated by Student's t test.

Effect of low level gallium arsenide exposure on few biochemical variables in rat kidney

Effect of gallium arsenide on kidney TBARS and glutathione levels is shown in Table 2 and 3. Kidney TBARS level more pronounced increased in gallium arsenide exposed animals after exposure of 6 months. Also, noted depletion of glutathione level on exposure to gallium arsenide.

DISCUSSION

The present study investigates the influence of low level GaAs on selected blood and tissue biochemical variables indicative of alteration in heme biosynthesis pathway and tissue oxidative stress at different time intervals. GaAs toxicity is dependent upon (a) route of exposure, (b) dissolution of compound, and (c) the size of particle, as smaller particle size dissolve more rapidly (Rosner and Carter, 1987). In the present study fine powder of GaAs was used to obtain maximum dissolution.

Determination of blood ALAD activity is an early indicator of GaAs toxicity (Flora et al., 1999). In the present study we observed maximum depletion of ALAD activity at month 1, 2 and 6 which showed maximum inhibition at month 6. These results could be attributed to the fact that maximum dissolution on GaAs into its constituents moieties occur after month 2 (Flora et al. 1998; Flora et al. 1997). It has been observed that Ga moiety is chiefly responsible for the haemato-toxic effects of GaAs leading to the inhibition of ALAD activity (Flora and Kumar, 1996). Relative sensitivity of ALAD to trivalent Ga (III) and As (III) has also been reported (Goering et al., 1998). The mechanism of GaAs toxicity is mainly due to dissociation of GaAs into Ga (OH)3 and AsH3 in acidic medium (Carter et al., 2003). Therefore, decreased ALAD activity on GaAs exposure might be due to the binding of As (III) to the essential –SH group present in the ALAD enzyme (Flora et al., 1998). Decrease in ALAD activity has been related to nutritionally inadequate diet, particularly deficient in zinc thus in the present study we selected zinc as possible antagonistic element in preventing GaAs induced inhibition of ALAD. Significant recovery in depleted ALAD activity was observed during concomitant zinc administration, which might be due to preferential binding of zinc to protein as zinc is required for various biochemical and physiological function in the body (Underwood, 1977). It has been reported that when water is placed in contact with GaAs surface, arsenic preferentially migrates to the surface where it gets oxidized and dissolved leaving behind solid Ga (Carter et al., 2003). It also suggests that mechanism of GaAs is mainly due to the presence of As (III). Goering et al., (Goering et al., 1988) also reported beneficial effect of zinc administration on depleted ALAD activity. The higher sensitivity of ALAD towards Ga relative to arsenite and arsenate has also been reported (Yamamuchi et al., 1989) suggesting gallium is the primary inhibitor of ALAD. We did not obtain any significant alterations in GSH concentration which is in accordance with our previous results (Flora et al., 1999). GSH is a tripeptide of three amino acids (Glutamic acid, Cysteine, Glycine) which is involved in the various physiological and metabolic functions. It is a major interorgan transporter of cysteine which offers a nucleophilic thiol group important for detoxification of electrophilic metabolites (Piomelli et al., 1987; Rana et al., 2002). Liver is considered as a glutathione generating factor which supplies to kidney and intestine with other constituents required for glutathione synthesis. In the present experiment decreased concentration of GSH in liver and kidney shows antioxidant action of GSH. (Flora et al., 1998) also reported significant decline in GSH concentration followed by GaAs exposure at month 6.

The hepatotoxic effects of GaAs would also be explained by the fact that there was simultaneous glutathione depletion. It is well known that depletion of GSH is considered to be a sensitive indicator of oxidative stress and from the present study it is clear that arsenic moiety in GaAs may be a causative factor for producing oxidative stress possibly due to disruption of prooxidant/antioxidant balance. GaAs induced TBARS production also support oxidative stress theory related to its exposure which is in accordance with present experimental findings. We also obtained maximum elevated concentration of TBARS at month 6

CONCLUSION

The present study thus led us to conclude that GaAs exerts its toxicity in time dependent manner which is mainly related to its dissolution and maximum toxicity occurring at month 6 however, after exposure was stopped there might be a recovery.

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