LETTERS TO THE EDITOR

Effects of Multiple Gallium Arsenide Exposure on Some Biochemical Alterations in Rat Brain

Key words: Gallium arsenide — Biogenic amines — Acetylcholinesterase activity — Heme synthesis

Gallium arsenide (GaAs), an intermetallic semiconductor is used primarily in the microelectronic industry. GaAs has been most often used for optoelectronic devices including light emitting diodes (LEDs), optical communication transmitter and solid state semiconductor laser etc. Exposure of workers to GaAs in the microelectronic industry can occur during preparation of materials, cleaning and maintenance operations for quartz glassware and during cleaning of reactor. The toxic effects from the intermetallic semi-conductor appear to occur after inhalation or oral exposure and may result in toxicity. Assessment of risk to workers engaged in GaAs production is difficult due to the lack of toxicity data for this compound. Its toxicity is mainly estimated on the basis of inorganic arsenic toxicity because it is well known that GaAs dissociates into its constitutive moieties *in vivo* and *in vitro* following exposure¹⁻³). Intratracheal administration of 30 mg/kg GaAs produced enzymatic changes in the body characteristic of soluble arsenic compounds; equivalent changes were observed when 1,000 mg/kg was given by oral administration¹). These results were all attributed to arsenic because no gallium was detected in either blood or urine. Few other recent studies have indicated that a marked inhibition of δ -aminolevulinic acid dehydratase (ALAD) occurs following dissolution of GaAs *in vivo*^{4, 5}). Changes in pulmonary tissue were also observed^{2, 5}) within a week after a single exposure to GaAs. There is very few report on the effects of GaAs on other extra-pulmonary tissues. Further, most of the previously reported data deal with effects after single exposure. The present study, thus, was planned to determine the dose dependent effects of GaAs on some selected brain biochemical variables following multiple oral exposure.

Male albino rats of wistar strain weighing approximately 120 g (5–6 weeks old) were obtained from Defence Research & Development Establishment animal facility and acclimatized for 7 days prior to dosing. Rats were allotted to four groups (n = 10) so that the group means and standard deviations in body weight were approximately equal. Rats received 50, 100 or 200 mg/kg GaAs orally through gastric intubation daily between 1,000 to 1,100 hrs 5 days a week (Monday to Friday) for 3 weeks. Control rats received a sham dose of vehicle by the same route. GaAs was suspended in phosphate buffer (0.1 M, pH 6.5) and delivered within 5 minutes from buffer addition. This procedure ensured that a minimal

amount of GaAs would be in soluble form at the time of administration. Rats were kept in individual cages. Body weight gain of rats in all the groups was monitored twice a week and any change in gross behavior of the exposed rats was also observed.

At the end of the 21st day of the study; rats were decapitated at 1,000 hrs on the 22nd day and the brain was rapidly removed and placed on ice. Blood was collected from heart in a separate tube for plasma separation. Half portion of the brain was homogenized in ice cold n-butyl alcohol and processed for biogenic amine determination⁶. Metabolites of dopamine (DA) and 5-hydroxytryptamine (5-HT), i.e., homovanillic acid (HVA) and 5 hydroxyindoleacetic acid (5-HIAA) were measured following the procedure of Haubrich and Denzer⁷. δ -aminolevulinic acid dehydratase (ALAD) activity in blood and brain was measured following the method of Gibson *et al.*⁸. Blood and brain glutathione (GSH) contents were determined as described by Jollow *et al.*⁹. The estimation of acetylcholinesterase (AChE) activity in plasma and brain was carried out according to the method of Ellman¹⁰ using acetylthiocholine as substrate. Measurement of arsenic in brain was carried out using atomic absorption spectrophotometer (AAS, Varian Techtron 30/40, Australia) fitted with a graphite furnace following wet acid digestion. Different brain areas were selected for histopathological evaluation in each animal. Brain was fixed in 10% buffered formalin. Standard brain section that permitted concurrent observation of each area was prepared. After processing, multiple sections from each block were prepared at 10–12 μ m thickness, stained with haematoxylin and eosin and examined under light microscope.

No significant change in the body weight and gross behavior between GaAs exposed and control animals was noticed (Data not shown). The administration of GaAs (200 mg/kg) resulted in a significant increase in norepinephrine (NE) level, while significant elevation of brain HVA (at all dose level) was observed (Table 1). Brain AChE activity increased significantly but not dose dependently, while significant inhibition of plasma AChE was noticed at all dose levels (Table 1).

Dose dependent inhibition of blood ALAD activity and moderate inhibition of brain ALAD (Table 2) occurred at the higher dose (200 mg/kg). Blood haemoglobin and packed cell volume remained uninfluenced on GaAs exposure. No significant change in brain GSH content was noticed following GaAs exposure at the lower dose levels while, it increased significantly on 200 mg/kg GaAs exposure. A dose dependent increase in blood GSH content was observed with a significant increase occurred at the highest dose only (200 mg/kg). Blood and brain As contents increased significantly with the increase in GaAs dose. Histopathological observations revealed GaAs at a dose level of 200 mg/kg produced mild necrosis and loss of neurons in cerebral cortex compared to normal control (Figs. 1 and 2). The lesions were focal but not symmetrical. These lesions were subtle characterized by nuclear necrosis and occasional vacuolation of the perikaryon (Figs. 1 and 2). No lesions were observed in hippocampus and cer-

Table 1. Dose dependent effects of gallium arsenide (50, 100 or 200 mg/kg, orally for 21 days) on brain biogenic amine level, their metabolites, brain and plasma acetylcholinesterase activity in rats.

	Brain						Plasma
	DA ^a	NE ^a	5-HT ^a	HVA ^a	5-HIAA ^a	AChE ^b	AChE ^b
Control	0.61 ± 0.02*	0.84 ± 0.03*	1.07 ± 0.07*	1.33 ± 0.02*	1.43 ± 0.06*	3.43 ± 0.51*	5.07 ± 0.33*
GaAs 50 mg kg ⁻¹	0.61 ± 0.02*	0.85 ± 0.02*	1.10 ± 0.03*	1.58 ± 0.03 [†]	1.49 ± 0.03*	11.74 ± 1.44 [†]	4.35 ± 0.37*
GaAs 100 mg kg ⁻¹	0.65 ± 0.02*	0.91 ± 0.01*	1.10 ± 0.03*	1.62 ± 0.01 [†]	1.74 ± 0.06*	12.26 ± 1.56 [†]	3.60 ± 0.24*
GaAs 200 mg kg ⁻¹	0.67 ± 0.04*	1.06 ± 0.01 [‡]	1.01 ± 0.03*	1.67 ± 0.03 [†]	1.72 ± 0.08*	7.17 ± 1.00 [‡]	3.53 ± 0.36*

a: µg g⁻¹, b: µmol-acetylthiocholine hydrolyzed min⁻¹ g-tissue⁻¹ or ml-plasma⁻¹

Values are mean ± S.E., n = 5.

*, †, ‡; Means with matching symbol notations within the same column are not different at the 5% level of significance. Data was analyzed by one-way analysis of variance followed by Dunnett's test.

Table 2. Dose dependent effects of gallium arsenide (50, 100 or 200 mg/kg, orally for 21 days) on some biochemical variables in blood and brain of rat.

	Blood					Brain		
	ALAD ^a	GSH ^b	As ^c	Hb ^d	PCV ^e	ALAD ^a	GSH ^b	As ^c
Control	6.59 ± 0.14*	53.9 ± 1.98*	0.03 ± 0.01*	13.3 ± 0.29*	44.7 ± 0.5*	2.77 ± 0.11*	5.38 ± 0.09*	N.D.
GaAs 50 mg kg ⁻¹	3.43 ± 0.21 [†]	55.3 ± 2.18*	1.47 ± 0.21 [†]	12.5 ± 0.24*	43.4 ± 0.9*	2.50 ± 0.13*	5.68 ± 0.15*	0.11 ± 0.007 [†]
GaAs 100 mg kg ⁻¹	2.92 ± 0.17 [†]	59.4 ± 1.55*	4.93 ± 0.31 [‡]	12.0 ± 0.18*	44.1 ± 0.4*	1.63 ± 0.06 [†]	5.96 ± 0.17*	0.19 ± 0.006 [‡]
GaAs 200 mg kg ⁻¹	1.61 ± 0.11 [‡]	69.2 ± 1.91 [†]	12.41 ± 2.07 [§]	12.0 ± 0.30*	42.6 ± 0.7*	1.01 ± 0.06 [‡]	6.14 ± 0.21 [†]	0.39 ± 0.006 [§]

N.D. denote 'not-detectable'

a: nmol min⁻¹ ml-erythrocyte⁻¹ or mg-protein, b: µ mol/100 ml blood or g-tissue, c: µg 100 ml blood or g tissue, d: g 100g⁻¹, e: % volume.

Values are mean ± S.E., n = 5.

*, †, ‡, §; Means with matching symbol notations within the same column are not different at the 5% level of significance. Data was analysed by ANOVA followed by Dunnett's test.

ebellum region.

The demand for high speed microelectronic devices for various modes of communications and military purposes has resulted in growing interest in alternatives to silicon with particular attention to compounds of group III–V of the periodic table. GaAs has proved to be an ideal substitute material for the above uses but is associated with unique health hazard. The recent increasing use of GaAs has

Fig. 1. Cerebral cortex from a male control rat brain. H&E X 100.

Fig. 2. Cerebral cortex from male rat brain exposed to gallium arsenide (200 mg/kg, orally for 3 weeks) showing shrunken mesotic nuclei of cells. H&E, X 100.

produced the need for the toxicological data on GaAs. The present results demonstrate for the first time the effect of multiple GaAs exposure on the steady state level on some brain neurotransmitters and a few other brain biochemical variables. Effect of GaAs on cholinergic neurotransmitter (acetylcholine, ACh) has been studied indirectly by the determination of its hydrolytic enzyme (AChE). The inhibitory role of GaAs on plasma AChE indicates the probable systemic increase in ACh level which might be protecting the animal from the deleterious effects of increased level of brain norepinephrine during GaAs exposure¹¹⁾. Thus, it appears that the whole body ACh increase might compensate the deleterious effects of brain norepinephrine increase even in the presence of brain AChE decrease, which is suggested by an increase in ACh activity in the brain. The results indicate only a moderate effect of GaAs on the level of brain biogenic amines while a marked influence on the brain and blood AChE activity. The present results will be useful as baseline data for further research in this area.

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*Division of Pharmacology & Toxicology, Defence
Research & Development
Establishment, Tansen Road Gwalior-474 002,
India*

**Swaran J.S. Flora, Shashi N.
Dube, Satish C Pant and
Amar S. Sachan**

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