Amelioration by Vitamin A upon Arsenic Induced Metabolic and Neurotoxic Effects

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Arsenic effect was studied at two dosage levels (0.5 mg and 1 mg/kg) for 45 days in two regions of brain, viz., cerebral hemisphere (CH) and cerebellum (C) in adult mice. This study included the antioxidant profile namely superoxide dismutase (SOD), catalase, lipid peroxidation, reduced glutathione, ascorbic acid and total sulphydryl groups. Adenosine triphosphatase (ATPase), succinic dehydrogenase (SDH), phosphorylase together with glycogen and protein levels were also estimated as metabolic indices in the brain regions of mice. A notable decrease was detected in the activities of the enzymes and in the levels of other metabolites together with a significant increase in the lipid peroxidation, glycogen and inorganic arsenic levels after arsenic administration. Supplementation of vitamin A to the arsenic treated mice brought about no significant variation in these antioxidant and metabolic indices in comparison to that of control, revealing amelioration by vitamin A on arsenic exerted metabolic and neurotoxic effects in mice.

Key words ——— arsenic, vitamin A, antioxidant system, metabolic and neurotoxic effect

INTRODUCTION

Arsenic, a heavy metal, an environmental toxicant and a ubiquitous trace element is found naturally in ground water and as a contaminant from industrial and agricultural use. Inorganic arsenic has been classified by IARC (International Agency for Reserch on Cancer, Geneva) as Group I carcinogen.[1] Groundwater arsenic contamination in Bangladesh and West Bengal has identified patients with arsenical skin lesions,[2] cancer of lungs, bladder etc.[3] Chronic exposure to humans has been associated with the disorders of the peripheral vasculature,[4] diseases of the cardiovascular system,[5] diabetes[6] and reproductive failure.[7] Arsenic has also been known to be neurotoxic.[8,9] Neurologic indications such as 'Pins and needles' or electric shock like pain in the lower extremities,[10] Guillian-Barre-Strohl syndrome (LGBS) etc. has been reported among Indian individuals exposed to elevated arsenic in drinking water.[11]

Inorganic arsenic (iAs) acts as a tumor promoter in part by inducing rapid burst of reactive oxygen species (ROS) in mammalian cells, resulting in oxidative stress[12,13] and carcinogenesis in man.[14] Studies document that arsenic generates ROS such as hydrogen peroxide, superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals.[13] These free radicals and the other reactive species are constantly produced in the brain *in vivo*.[15] Experiments on human fetal brain explants on exposure to arsenic in culture have showed disturbances in lipid peroxidation, generation of ROS and apoptosis.[16] Adenosine triphosphatase (ATPase) and succinic dehydrogenase (SDH) (enzymes of the inner mitochondrial membrane) are concerned with respiration, and energy generation; hence, are essential for cell survival. Respiratory chain is the major source of ROS, hence mitochondrial oxidative damage is thought to be a proximal cause of metabolic effects.[17,18] Metabolism in fish brain was also affected by arsenic poisoning[19] in addition to other pathological effects in general metabolism of animal tissues.[20]

Oxidative damage in mice brain by arsenic treatment from our laboratory has earlier been reported by Rao and Avani.[8] However, our literature survey yielded scanty reports on the use of vitamin A to combat arsenic toxicity. Hence, this study was undertaken to assess the possible beneficial effects of this vitamin upon arsenic induced neurotoxicity.

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MATERIALS AND METHODS

Animals ——— Healthy, adult male albino mice (*Mus musculus*) of Swiss strain weighing between 30–40 g were procured from Alembic Pharmaceuticals, Vadodara, Gujarat, India under the Animal Maintenance and Registration No. 167/1999/CPCSEA from the ministry of Social Justice and Empowerment, Government of India. All the animals were acclimatized seven days prior to the commencement of the treatment. They were housed in air-conditioned animal house at a temperature of 26 ± 2°C and exposed to 10–12 hr of daylight and a relative humidity of 30–70%. Animals of different groups were caged separately and were maintained on standard chow [National Institute of Occupational Health (NIOH) containing wheat –70%, gram –20%, fish meat –5% and yeast powder –5%] and water was given *ad libitum*.

Chemicals and Dose Selection ——— Arsenic trioxide (As_2O_3) was purchased from HiMedia Laboratories Ltd., Mumbai, India, and vitamin A (Aqua sol capsules) was obtained from USV Ltd., Mumbai, India. Stock solution of arsenic trioxide was prepared in double distilled water and orally given to mice with a hypodermic syringe. Both the doses for arsenic are derived from its LD_{50} value (39.4 mg/kg body wt; Harrison *et al*.[21] Hence, low dose is $1/80^{th}$ of LD_{50}; while high dose is $1/40^{th}$ of LD_{50}. The dose for vitamin A is based on earlier work done in our laboratory.[22] Both the treatments were given seperately (arsenic in the morning and vitamin A in the evening) so as to avoid their interaction.

Experimental Groups ——— Animals were divided into following six groups: Group I served as untreated (control) animals, Group II were orally given 0.5 mg/kg by.wt As_2O_3 (low dose — LD) while Group III were orally treated with 1 mg/kg by.wt As_2O_3 (high dose — HD). Group IV were given 0.2 mg/kg by.wt vitamin A alone. Animals in group V were orally administered 0.5 mg As_2O_3/kg + 0.2 mg/kg by.wt of vitamin A and group VI were orally given 1 mg As_2O_3/kg by.wt + 0.2 mg/kg body weight vitamin A.

All the groups were treated for 45 days period. All the treated mice were alive before the autopsy. At the end of each treatment, the animals were weighed and then sacrificed using light ether anesthesia. The cerebral hemisphere (CH) and cerebellum (C) of mice brain were dissected out carefully, blotted free of blood and weighed up to the nearest milligram and used for the study. For all biochemical estimations, a minimum of 8–10 replicates was done for each parameter and tissue.

Body and Organ Weights ——— The body weight of control and all treated groups of mice were recorded to the nearest milligram on an animal weighing balance. Similarly, weights of both cerebral hemisphere and cerebellum were recorded to the nearest milligram on digital balance (Citizen, Japan).

Biochemical Analysis ———

Superoxide dismutase (SOD) (E.C.1.15.11): The activity of SOD was assayed by the modified spectrophotometric method of Kakkar *et al*.[23] In the assay system, control consisted of 2.4 ml phosphate buffer (0.052 M), 0.1 ml phenazine methosulphate (186 μmol), 0.3ml 0f nitroblue tetrazolium chloride (30 μmol), 0.2 ml fresh NADH (780 μmol). To the sample system 0.2 ml of enzyme was added prior to the addition of NADH. The reaction was stopped by addition of 1 ml of acetic acid. Four ml of *n*-butanol was then added and shaken vigorously. The tubes were centrifuged for 10 min at 2000 rpm. The optical density was measured at 560 nm. Its activity was measured as units SOD/mg protein (One unit of SOD enzyme activity is defined as the enzyme concentration required to inhibit 50% of OD (optical density) of chromogen formed in 1 min at 560 nm under assay condition).

Catalase (E.C. 1.11.1.6): The activity of catalase was assayed by the modified method of Luck.[24] The assay mixture consisted of 0.5 ml 50 mM phosphate buffer, pH 7.0 and 1 ml aliquot. The blank readings were subtracted from the test readings for obtaining correction factor for the spontaneous reaction. The decrease in absorbance was noted every 5 sec for 30 sec at 240 nm. The enzyme activity was expressed as μmol of H_2O_2/mg protein.

Lipid Peroxidation (LPO): LPO was determined by the method of Ohkawa *et al*.[25] To the sample tube containing 0.1 ml distilled water, 0.75 ml of 20% acetic acid, 0.2 ml sodium dodecyl sulphate (SDS), 0.75 ml of 1% thiobarbituric acid (TBA), and 0.1 ml of homogenate was added. The blank tube contained 0.1 ml distilled water instead of homogenate. The solutions were mixed and heated in a water bath for 60 min at 95°C. The tubes were cooled, mixed, and centrifuged at 1000 *g* for 15 min. The OD of the supernatant was read at 532 nm and was expressed as nmoles malonyldialdehyde (MDA) formed/100 mg tissue wt.

Reduced Glutathione (GSH): Glutathione was estimated by the method of Grunert and Philips.[26] A known amount of tissue was homogenized in 3 ml

3% metaphosphoric acid (HPO_3) and 1 ml distilled water and saturated with salt solution (1.5 g NaCl crystals). The tubes were centrifuged at 800 g for 5 min. 2 ml aliquot of supernatant was added to the sample tube containing 6 ml saturated NaCl solution and allowed to stand for 10 min at 20°C.The blank tube was run with 2 ml of 2% HPO_3 instead of the supernatant aliquot. Sodium nitroprusside and sodium cyanide, 1 ml each, was added to the blank and sample tubes. The optical density was measured at 520 nm and the levels were expressed as μg glutathione/100 mg fresh tissue wt.

Total, Dehydro and Reduced Ascorbic Acid (TAA, DHA and RAA): Ascorbic acid was determined by the method of Roe and Kuether[27] The tissue homogenate was prepared in 10 ml Norit reagent. The mixture was shaken well and allowed to stand for 15 min and then filtered through Whatman filter paper No. 42. To 4 ml of homogenate, 1 ml of 2,4-dinitrophenyl-hydrazine reagent was added and then a drop of 10% thiourea was added in order to activate the reaction. Blank tube was run with 4 ml of 6% trichloroacetic acid (TCA) instead of homogenate and the standard tube with 4 ml ascorbic acid solution (10 μg/ml). The tubes were kept in boiling water bath for 15 min and cooled in ice bath. 5 ml of sulphuric acid was added along the sides of the tubes. The tubes were allowed to stand for 30 min and the optical density of the colour was measured at 540 nm against blank. Its levels were expressed in mg/g tissue wt. The dehydro form was estimated by using 6% trichloroacetic acid. The difference between total and dehydro-ascorbic acid gave the value of RAA. Its levels were expressed in mg/g tissue wt.

Total Sulphydryl Groups (-SH): Total -SH groups were estimated by modified method of Sedlak and Lindsey[28] and were expressed as mg/g tissue wt. 0.5 ml homogenate (made in 0.02 M Tris EDTA) was mixed with 1.5 ml of 0.2 M Tris EDTA buffer and 0.01 M 5′,5′-dithiobis-2-nitro benzoic acid (DTNB). The mixture was made up to 10 ml with 7.9 ml of absolute methanol. A reagent blank (without sample) and a sample blank (without DTNB) were prepared in a similar manner. The tubes were allowed to stand for 15 min and then centrifuged for 15 min at 3000 *g* and the respective supernatants were read at 412 nm. The serum was suitably diluted with 0.02 M Tris EDTA for determining total -SH groups.

Total Proteins: Protein levels were estimated by the method of Lowry *et al*.[29] and expressed as mg protein/100 mg tissue. To 0.2 ml of tissue homogenate in distilled water, 0.6 ml of distilled water and 4 ml of alkaline $CuSO_4$ solution were added, mixed and incubated at room temperature for 15 min. Phenol reagent (0.4 ml) was added, mixed and the preparation was incubated for 30 min at room temperature. The colour intensity was measured at 540 nm. The same method was used for the estimation of protein in the serum after diluting it suitably with distilled water.

ATPase (E.C.3.6.1.3): The ATPase activity was assayed by the method of Quinn and White[30] while inorganic phosphate liberated was estimated using the method of Fiske and Subbarow.[31] The reaction mixture contained 0.3 ml substrate buffer [3 mM ATP disodium salt in Tris hydrochloride (HCl) buffer], 0.1 ml $MgCl_2$ (3 mM), 0.1 ml NaCl (150 mM), 0.1 ml potassium chloride (KCl) (30 mM) followed by 0.2 ml of tissue homogenate and 0.2 ml tris sucrose buffer. In the blank tube, 0.3 ml of distilled water was taken instead of substrate buffer. The contents of the tube were thoroughly mixed and incubated at 37°C for 30 min. For termination of the reaction, 0.5 ml 10% TCA was added to the tubes and they were kept for precipitation at 4°C for 15 min. The solution was centrifuged for 3000 rpm for 15 min. The clear supernatant fluid was used for determination of inorganic phosphate. Readings were taken at 660 nm. The enzyme activity was expressed as μmol of inorganic phosphate released/mg protein/30 min.

Succinate Dehydrogenase (SDH) (E.C.1.3.9.9): The activity of SDH was estimated according to the method of Beatty *et al*.[32] To sample tubes containing 0.4 ml of tissue homogenate in cold distilled water, 1 ml 0.2 M phosphate buffer, 1 ml 0.1 M sodium succinate (substrate), and 1 ml 2,4-iodophenyl-3,4-nitrophenyl-3,4-nitrophenyl-5-phenyl tetrazolium chloride (INT) was added and incubated for 15 min at 37°C. 1 ml distilled water was added to the blank tube instead of INT solution. The reaction was terminated by 0.1 ml of 30% TCA. The resultant formazan was extracted in 7 ml ethyl acetate and colour intensity was measured at 420 nm against blank. The activity was expressed as É g formazan/100 mg fresh tissue weight.

Phosphorylase (E.C. 2.4.1.1): The activity of enzyme phosphorylase was assayed by the method of Cori *et al*.[33] The inorganic phosphorous liberated was determined by method of Fiske and Subbarow.[31] 0.1 ml of tissue homogenate was added to a cold incubation mixture containing 0.2 ml sodium citrate buffer (0.1 M; pH 5.9), 0.3 ml of potassium fluoride (0.15 M) and 0.05 ml of glucose-1-phosphate (0.2 M

Table 1. Gravimetric and Reactive Oxygen Species in CH and C in Arsenic and Vitamin A Exposed Mice

PARAMETER	ORGAN	CONTROL	LD	HD	CONTROL	LD	HD
			As_2O_3			Vitamin A	
Organ weight/	CH	9.49	9.60	10.38	9.61	9.58	9.93
Body weight	C	3.02	2.97	2.85	3.26	3.10	3.14
SOD	CH	12.02 ± 0.17	8.22 ± 0.95*	4.70 ± 0.36*	12.09 ± 0.67	11.75 ± 1.16^{NS}	11.66 ± 1.37^{NS}
(units/mg		(100)	(68)	(39)		(98)	(97)
protein)	C	11.62 ± 1.14	7.91 ± 1.44	4.83 ± 0.81*	11.81 ± 0.30	10.90 ± 0.92^{NS}	10.81 ± 0.94^{NS}
		(100)	(68)	(42)		(94)	(93)
Catalase	CH	44.18 ± 4.58	35.62 ± 1.85*	28.02 ± 3.96*	48.57 ± 5.31	43.61 ± 6.88^{NS}	43.53 ± 8.59^{NS}
(μmol H_2O_2		(100)	(81)	(63)		(99)	(98)
consumed/mg	C	42.63 ± 2.72	38.62 ± 3.72**	35.65 ± 3.76*	43.55 ± 2.88	42.42 ± 1.34^{NS}	41.82 ± 4.66^{NS}
protein/minute)		(100)	(91)	(84)		(99)	(98)
Lipid Peroxidation	CH	27.69 ± 0.84	40.42 ± 0.67*	72.6 ± 1.37*	26.89 ± 1.99	27.05 ± 1.19^{NS}	27.02 ± 1.43^{NS}
(nanomoles of MDA/		(100)	(146)	(262)		(98)	(97)
mg tissue weight/	C	27.95 ± 1.87	45.29 ± 4.62*	68.13 ± 2.52*	27.32 ± 2.55	27.56 ± 1.31^{NS}	27.31 ± 1.27^{NS}
60 min)		(100)	(162)	(244)		(99)	(98)
Glutathione	CH	60.54 ± 5.29	38.5 ± 0.43*	35.28 ± 1.47*	58.02 ± 3.63	60.13 ± 3.6^{NS}	59.54 ± 2.72^{NS}
(μg/100 mg		(100)	(64)	(58)		(99)	(98)
tissue weight)	C	67.88 ± 9.02	37.53 ± 1.19*	49.03 ± 1.99*	66.82 ± 2.4	65.72 ± 3.08^{NS}	64.45 ± 3.3^{NS}
		(100)	(55)	(49)		(97)	(95)

*$p < 0.001$, **$p < 0.01$, NS = Not Significant. LD = Low Dose, HD = High Dose. Values are Mean $\pm$ S.D., Figures in parenthesis indicate percent values.

disodium salt). The solutions were incubated for 15 min at 37°C after which 1 ml of 10% TCA was added to stop the enzyme activity. The incubated solutions were then centrifuged and the supernatant was utilized for estimation. The tubes were then placed in cold water bath at 20°C. For control, 1 ml of TCA was added in place of homogenate. Then 1 ml of ammonium molybdate solution was added to all the tubes followed by 0.4 ml of 1, 2, 4 aminonapthol sulphonic acid reagent (ANSA) and the total volume was made up to 10 ml with distilled water. The solutions were mixed and allowed to stand for 6 min and the optical density of the blue colour thus developed was read at 660 nm. The enzyme activity was expressed as μmol inorganic phosphate released/100 mg fresh tissue weight/15 min.

Glycogen: Glycogen levels were estimated using the method of Seifter *et al*.[34] A known amount of tissue was digested in 2 ml of 30% boiling potassium hydroxide (KOH) solution followed by washing with ethanol. The digest was extracted in ethanol, precipitated at 4°C and dissolved in known volume of distilled water. 1 ml of aliquot of this sample was added to 4 ml of anthrone reagent and kept in boiling water bath for 4 min. After cooling, the resultant colour was read at 620 nm and expressed as μg/100 mg tissue weight.

Inorganic Arsenic Estimation: Arsenic retention in brain and whole blood was estimated by Arsenic Test (sensitive) kit (Analytical Test Strip method) obtained from Merck, Germany (Cat. No. 1.17926.0001).

Statistical Analysis —— Data was statistically evaluated using Student's *t*-test and analysis of variance (ANOVA). Percent values were also calculated. All the experimental groups were compared with control group. Comparison of vitamin A alone with control showed no significance.

RESULTS

Organ Weight/Body Weight

Ratio of organ/body weights showed no significant variations (Table 1).

SOD

The SOD levels showed a significant decline ($p < 0.001$) in its activity in both the cerebral hemisphere and cerebellum regions of brain after low and high doses of arsenic treatment. As evident from the percent values, in vitamin A-supplemented groups,

Table 2. Ascorbic Acid in CH and C in Arsenic and Vitamin A Exposed Mice

ORGAN	CONTROL	LD	HD	CONTROL	LD	HD
		As_2O_3			Vitamin A	
CH	5.34 ± 0.49	4.39 ± 0.19**	4.23 ± 0.11***	5.40 ± 0.59	5.03 ± 0.65^{NS}	4.67 ± 0.50^{NS}
	(100)	(82)	(79)		(94)	(87)
C	4.81 ± 0.50	3.60 ± 0.70**	2.56 ± 0.23*	5.41 ± 0.51	4.59 ± 0.40^{NS}	4.34 ± 0.91^{NS}
	(100)	(75)	(53)		(95)	(90)
CH	1.29 ± 0.10	2.52 ± 0.19**	2.69 ± 0.18*	1.32 ± 0.16	1.46 ± 0.16^{NS}	1.72 ± 0.17^{NS}
	(100)	(195)	(209)		(113)	(133)
C	1.31 ± 0.11	2.10 ± 0.21**	2.23 ± 0.12**	1.23 ± 0.19	1.24 ± 0.10^{NS}	1.77 ± 0.50^{NS}
	(100)	(161)	(171)		(95)	(135)
CH	4.05 ± 0.44	1.87 ± 0.72*	1.59 ± 0.20*	4.08 ± 0.54	3.57 ± 0.43^{NS}	3.09 ± 1.23^{NS}
	(100)	(46)	(39)		(88)	(76)
C	3.50 ± 0.35	1.50 ± 0.69**	1.58 ± 0.36***	4.19 ± 0.36	3.35 ± 0.36^{NS}	2.56 ± 0.69^{NS}
	(100)	(61)	(43)		(96)	(99)

*$p < 0.001$; **$p < 0.05$; ***$p < 0.01$, NS = Not Significant. LD = Low Dose, HD = High Dose. Values are Mean ± S.D., Figures in parenthesis indicate percent values.

there was considerable revival and the values remained unchanged with respect to control group (Table 1).

Catalase

Catalase activity demonstrated a highly significant decline ($p < 0.001$) after arsenic trioxide treatment at both dosages in cerebral hemisphere. Similarly, in cerebellum a significant reduction ($p < 0.05$ and $p < 0.01$) was observed after arsenic exposure. However, with co-administration of vitamin A its values were comparable to that of control as evident from the percentage values (Table 1).

LPO

The lipid peroxidation levels recorded a steep elevation ($p < 0.001$), in cerebral hemisphere and cerebellum at both the dosages of arsenic. However, in the vitamin A-supplemented groups, both the brain portions showed a significant comparison with that of control (Table 1).

GSH

This parameter showed a remarkable reduction ($p < 0.001$) in cerebral hemisphere and cerebellum regions. With co-administration of vitamin A these values revealed a marked comparison with the untreated group. Same was noted for percent values (Table 1).

Total, Dehydro and Reduced Ascorbic Acid Levels (TAA, DHA, and RAA Levels)

Arsenic treatment brought about a remarkable ($p < 0.01$; $p < 0.001$) depression in the TAA levels, with an escalation ($p < 0.001$) in the DHA levels. RAA also noted a significant ($p < 0.001$) decrease in both arsenic treated groups in cerebral hemisphere. In cerebellum alteration of $p < 0.01$ and $p < 0.001$ was noted. Its percent values recovered in the vitamin A supplemented groups (Table 2).

Total Protein and Total Sulphydryl Groups

Both these parameters in brain and serum showed a significant alteration ($p < 0.001$) after arsenic trioxide treatment. In the vitamin A supplemented groups, marked recuperation in CH and C regions of brain was observed (Table 3).

ATPase and SDH

Both the brain regions revealed a substantial depletion ($p < 0.001$) in the activities of ATPase and SDH enzymes. But, in vitamin A supplemented groups V and VI considerable reformation was brought about in both the enzyme activities as evident from the percent values (Table 4).

Phosphorylase and Glycogen

Arsenic trioxide treatment after 45 days severely declined the phosphorylase activity ($p < 0.001$), contrary to the steeply increased glycogen levels. In vitamin A-supplemented groups a significant recovery was observed in these parameters. Percent values also exhibited the same trend (Table 4).

Arsenic Levels

Control untreated mice (group I) and vitamin A

Table 3. Metabolic Parameters in CH and C in Arsenic and Vitamin A Exposed Mice

PARAMETER	ORGAN	CONTROL	LD	HD	CONTROL	LD	HD
			As_2O_3			Vitamin A	
Total -SH groups	CH	5.58 ± 0.79	3.52 ± 0.55*	2.49 ± 0.39*	5.91 ± 0.64	5.57 ± 0.66^{NS}	5.56 ± 0.54^{NS}
(mg/100 mg		(100)	(63)	(45)		(99)	(99)
tissue weight)	C	6.37 ± 0.28	3.10 ± 0.26*	2.28 ± 0.36*	6.33 ± 0.36	6.34 ± 0.29^{NS}	6.11 ± 0.66^{NS}
		100)	(49)	(36)		(99)	(96)
	SERUM	1.81 ± 0.18	0.70 ± 0.07*	0.43 ± 0.06*	2.00 ± 0.32	1.76 ± 0.26^{NS}	1.74 ± 0.17^{NS}
	(mg/ml)	(100)	(39)	(24)		(97)	(96)
Total proteins	CH	6.73 ± 0.19	4.55 ± 0.53*	2.74 ± 0.72*	6.70 ± 0.63	6.51 ± 0.49^{NS}	6.46 ± 0.28^{NS}
(mg/100 mg		(100)	(68)	(41)		(97)	(96)
tissue weight)	C	6.44 ± 0.19	4.88 ± 0.44*	3.34 ± 0.73*	6.77 ± 0.45	6.37 ± 0.24^{NS}	6.26 ± 0.17^{NS}
		(100)	(76)	(52)		(99)	(97)
	SERUM	18.88 ± 0.89	15.44 ± 0.60*	10.41 ± 1.10*	18.90 ± 1.26	18.61 ± 0.59^{NS}	18.40 ± 0.84^{NS}
	(mg/ml)	(100)	(82)	(55)		(99)	(97)

*$p < 0.001$, NS = Not Significant. LD = Low Dose; HD = High Dose. Values are Mean ± S.D. Figures in parenthesis indicate percent values.

Table 4. Carbohydrate Metabolism Parameters in CH and C in Arsenic and Vitamin A Exposed Mice

PARAMETER	ORGAN	CONTROL	LD	HD	CONTROL	LD	HD
			As_2O_3			Vitamin A	
(ATPase)[a]	CH	3.9 ± 0.20	2.85 ± 0.26*	2.41 ± 0.30*	4.19 ± 0.50	3.85 ± 0.30^{NS}	3.8 ± 0.19^{NS}
		(100)	(73)	(62)		(99)	(97)
	C	4.62 ± 0.46	3.15 ± 0.28*	2.57 ± 0.31*	4.65 ± 0.18	4.58 ± 0.47^{NS}	4.51 ± 0.71^{NS}
		(100)	(68)	(56)		(99)	(97)
(SDH)[b]	CH	56.26 ± 4.12	37.77 ± 2.89*	43.76 ± 1.69*	58.60 ± 8.12	55.42 ± 6.64^{NS}	55.21 ± 1.56^{NS}
		(100)	(67)	(44)		(98)	(98)
	C	68.79 ± 7.51	30.32 ± 5.16*	22.61 ± 3.03*	70.57 ± 5.57	68.06 ± 5.11^{NS}	67.73 ± 3.15^{NS}
		(100)	(44)	(33)		(99)	(98)
Phosphorylase[c]	CH	118.42 ± 8.91	60.71 ± 4.29*	35.43 ± 4.52*	119.12 ± 5.33	117.97 ± 5.26^{NS}	116.81 ± 5.98^{NS}
		(100)	(51)	(30)		(99)	(98)
	C	118.63 ± 7.68	54.27 ± 3.95*	24.68 ± 4.94*	120.85 ± 3.30	117.97 ± 11.13^{NS}	117.04 ± 5.77^{NS}
		(100)	(46)	(25)		(99)	(99)
Glycogen[d]	CH	1873 ± 66.44	1958.16 ± 60.33*	2141.95 ± 57.27*	1834.46 ± 51.21	1847.19 ± 77.26^{NS}	1842.26 ± 51.93^{NS}
		(100)	(104)	(114)		(99)	(98)
	C	1833.95 ± 81.98	1935.11 ± 74.59*	2102.85 ± 102.170*	1820.95 ± 50.04	1813.35 ± 101.78^{NS}	1800.12 ± 71.30^{NS}
		(100)	(105)	(109)		(98)	(98)

a) μmol i.p. released/mg protein/30 min. *b*) μg formazan released/15 min/mg protein. *c*) μg phosphorous released/mg protein/15 min. *d*) μg/100 mg tissue weight. *$p < 0.001$, NS = Not Significant. LD = Low Dose; HD = High Dose. Values are Mean ± S.D. Figures in parenthesis indicate percent values.

alone group (IV) showed null arsenic levels in the tissue and whole blood. In both — cerebral hemisphere and cerebellum regions of brain, low and high dosed animals showed arsenic deposition, which was also dose dependent. In vitamin A treated groups (V and VI), arsenic retention declined, and levels were comparable to control (Table 5).

DISCUSSION

A marked reduction in the body and organ weights of the arsenic treated mice was evident. Organ to body weight ratio was also calculated. In support of our data, decreased body and brain weights and food consumption in male Wistar rats were also confirmed by Nagaraja and Desiraju,[35] Wagstaff.[36] These reduced gravimetric values thus indicated the lethal nature of the toxicant.[37]

In the present study, levels of glutathione, ac-

Table 5. Arsenic Retention in Arsenic and Vitamin A Exposed Mice

ORGAN	CONTROL	LD	HD	CONTROL	LD	HD
		As_2O_3			Vitamin A	
CH & C (μg/100 mg tissue weight)	0.0	0.01	0.05	0.0	0.005	0.01
whole blood (μg/ml)	0.0	0.05	0.25	0.0	0.025	0.05

LD = Low Dose; HD = High Dose. Analytical Test Strip method purchased from Merck.

tivities of catalase and SOD were significantly decreased, whereas lipid peroxide levels were enhanced in the brain of adult mice after treatment with arsenic trioxide, which is in agreement with the earlier reports.[16,38,39] Because of high adenosine triphosphate (ATP) demand, brain consumes O_2 rapidly and is thus susceptible to interference with mitochondrial function, which can in turn lead to increased $\bullet O_2^-$ formation. Study conducted by Chaudhuri *et al*.[38] has also reported decreased SOD levels on rat brain exposed to arsenic in drinking water. Recent studies on the mechanisms of arsenic toxicity report participation of hydroxyl radicals in arsenic-induced disturbances in the CNS.[12] Trivalent arsenicals react *in vitro* with thiol containing molecules such as glutathione, cysteine or hemoglobin forming As-SH complex or $(GS)_3As(III)$ (with GSH)[16,38,39] and this property is considered to be the mechanism of action by which arsenic exerts its toxic effects. Arsenic generated ROS also attack -SH groups thus leading to their oxidation, thus damaging proteins and enzymes requiring -SH groups. However, the protection of -SH groups/GSH by vitamin A as evident from the stated data could be due to its ability to scavenge the oxygen radicals consequently preventing the free radical attack. Grosse *et al*.[40] have indicated vitamin A as an efficient $\bullet O_2^-$ scavenger; infact the antioxidant action of carotenoids are based on their singlet oxygen quenching property, which is also its best-documented action.[41] Several studies have also reported vitamin A to be an effective lipid antioxidant.[42–44] Chaudhuri *et al*.[35] and Chattopadhyay *et al*.[16] have reported increased lipid peroxidation at different levels of arsenic contaminated drinking water in rat brain and human fetal brain explants respectively. The decreased total ascorbic acid (TAA), levels in our study, are concomitant with that of Ramanathan *et al*.[45–47] who have also reported reduced ascorbic acid levels in arsenic intoxicated rats. Under arsenic induced stress, ascorbic acid gets rapidly oxidized and gets converted to the dehydro form, resulting in its increased form. Subsequently, DHA did not get converted to the reduced form (RAA) due to lack of GSH. Hence, increase in the DHA levels are reported after arsenic treatment. Earlier work in our laboratory documented comparable results in brain of mice.[39] Thus, arsenic probably causes disturbances in utilization and metabolism of ascorbic acid in the brain.

A decrease in the protein levels occurred following arsenic exposure, with a marked decrease especially in the high dosed groups. Reduction in protein levels could be attributed to their damage by singlet oxygen, often due to oxidation of essential amino acids.[48] Interaction between As(III) and thiol containing proteins and peptides have generally been regarded as basis for the effects of arsenic on structure and function of these molecules.[18] Aldehydes (MDA) formed during lipid peroxidation can react with -SH groups of proteins to damage them, thus inhibiting enzymes requiring -SH groups for their activities.[48] Thus, damage to proteins and total -SH occurs not only through ROS but also by binding of arsenite to these molecules. Further, arsenic trioxide treatment also inhibited SDH and ATPase enzymes in our study affecting neural tissue metabolism. Arsenic affects mitochondrial enzymes and impairs tissue respiration, which seems to be related to cellular toxicity of arsenic. Thus, reduction in total protein is probably attributed to loss of growth due to low food intake as a result of As^{+3} poisoning.

Arsenic inhibited the activities of carbohydrate metabolism enzymes — ATPase, SDH and phosphorylase; while increased the deposition of glycogen in the brain tissue of mice. Such results have been reported by Chinoy[49] of our laboratory after mice were exposed to arsenic trioxide. Arsenite inhibits SDH activity and also uncouples oxidative phosphorylation thereby decreasing ATP content in cell.[50] Shobha Rani *et al*.[19] have also reported a decrease in SDH activity in fish brain, in support of our data. Repetto *et al*.[51] have further documented inhibition of SDH activity by As(III). Mitochondria accumulate arsenic and respiration mediated by NAD-linked

substrates are particularly sensitive to arsenic and is thought to result from a reaction between arsenite ion and dihydrolipoic cofactor.[52] All these noxious effects on metabolites and antioxidants seemed to be correlated to the gradual retention of arsenic in brain regions. This is further demonstrated by loss of body and organ weights gradually with arsenic retention in brain tissue as reported earlier.[8,37]

Co-administration of vitamin A to As^{3+} exposed groups, these metabolic and antioxidant indices did not exhibit variation with those of control mice revealing its protective role. Retinoid is one of the most promising substances for chemoprevention against certain cancers. In the present study, when vitamin A (as retinyl palmitate) was supplemented along with both — low and high doses of arsenic, a remarkable resurgence was observed in the body and organ weights, antioxidant enzymes — SOD and catalase, together with the levels of glutathione, lipid peroxidation, and ascorbic acid. Brain and serum levels of protein, total -SH groups and the carbohydrate metabolism indices (ATPase, SDH, phosphorylase and glycogen) along with arsenic retention in the vitamin A supplemented groups were comparable to the control value. The antioxidant activity of vitamin A against lipid peroxidation induced by other test chemical in rat tissues *in vivo* is known.[43,44,53] This vitamin also acts as an antioxidant by decreasing peroxidation products, scavenging reactive oxygen species and inhibiting the activation of promutagen.[54] *In vivo* mitigating studies of doxorubicin by vitamin A (as retinol palmitate) in rat brain have indicated increased resistance of membrane lipids to peroxidation, both endogenously produced and induced *in vitro*. These results indicate that vitamin A may act as a physiological antioxidant in cell membranes where it is localized.[53] Grosse *et al*.[40] have indicated vitamin A as an efficient $\bullet O_2^-$ scavenger; infact the antioxidant action of vitamin A is based on their singlet oxygen quenching property, which is also its best documented action.[41] It can thus be proposed that scavenging of free radicals by vitamin A could lead to the protection of -SH groups, thereby the proteins, enzymes; and glutathione, as evident from these data.

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