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Effect of high arsenic content in drinking water on rat brain

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The permissible limit of arsenic content in drinking water is 0.05 ppm, whereas, in many parts of West Bengal the arsenic level in drinking water is 0.1 ppm, frequently 0.3 ppm and even 3.0 ppm, though rarely. In order to assess possible risk to brain function by drinking such water, rats were given arsenic mixed in drinking water at the above four concentrations for 40 days. There was increased lipid peroxidation at all doses of arsenic, including the 'permissible limit', decrease in glutathione level, superoxide dismutase and glutathione reductase activities, indicating the free-radical-mediated degeneration of brain.

Arsenic (As) is a naturally occurring element and is used as poison[1]. Ingestion of As is known to cause skin cancer and inhalation causes lung cancer[2]. Recently it has been reported that ingested As also causes cancer of the bladder, kidney, lung and liver[3]. Ramos *et al.*[4] have shown that As decreased glutathione (GSH) level and increased lipid peroxidation (LPO) in liver, kidney and heart. This effect was higher at 18.2 mg/ kg dose (a dose within the 95% confidence limit for LD_{100}) than that at 14.8 mg/ kg dose (a dose within the 95% confidence limit for LD_0). It has been reported[5] that when GSH is low As toxicity was enhanced. Therefore it is expected that a relationship between As toxicity, lipid peroxidation and GSH level exists.

LPO has been implicated in the pathology of various degenerative disorders of the central nervous system (CNS). Brain is thought to be particularly vulnerable to damage because of its high rate of oxygen consumption, the high levels of polyunsaturated fatty acids, low levels of peroxisomal catalase and glutathione peroxidase (GSG-Px) activities, low levels of mitochondrial superoxide dismutase and catalase activities, dependence of neurons on glial cells for synthesis of Cys from Cys-Cys and non regenerative nature of neuron. Lipid peroxidation in brain differs from liver system and uses either NADPH or NADH as the reducing source and only Fe^{2+} ion, whereas liver lipid peroxidation uses only NADPH as reducing source and both Fe^{2+} and Fe^{3+} ion[6]. We had earlier reported that As at the level known as "permissible limit" is harmful to brain[7,8].

Arsenic contamination of drinking water in West Bengal has been noted[9] as a serious health hazard as people suffer from melanosis, leukomelanosis, keratosis, hyperkeratosis, gangrene, skin cancer and other types of cancer. According to the report by Mondal *et al.*[10], more than 200,000 people of 520 villages covering 34,000 sq.km. area suffer from As related diseases. They have analysed 25,000 tube wells and found average As concentration to be approximately 0.3 mg/l and in some places the concentration reaches to 3.0 mg/l, whereas, 0.05 mg/l of As is the permissible limit of As in drinking water. People drinking As contaminated water have high As concentration in their hair, nails and urine[10]. But there are no information on the effect of that high concentration of As in brain. Therefore, in the present study an attempt was made to evaluate the effect of As in the brain of mammals at dose levels that were found contaminating the ground water and also at the "permissible limit" level laid by WHO. The parameters studied were GSH depletion, LPO and the activities of SOD, catalase, GSH-Px and GSH-red.

Materials and Methods

Sprague Dawley male rats (four weeks old; average body wt. 110 g) were divided into five groups. The first group (control) was given distilled water; while the second, third, fourth and fifth groups were given water containing As, 0.05 ppm (the "permissible limit"), 0.10 ppm, 0.30 ppm (the average concentration found in affected areas of West

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Bengal), and 3.0 ppm (the maximum levels found) respectively in the form of Na_3AsO_4 (E.Merck) in drinking water daily for forty days, with normal food *ad libitum*. They were kept in 12 hr light and 12 hr dark cycles in controlled temperature and humid condition.The volume of water intake was noted each day for all the groups. In our experiments young growing animals were used so that the changes in brain due to As can be studied with minimum interference from any possible age-related degenerative changes.

From earlier time course experiments it had been noted that neurotoxic effects in brain appeared after 40-60 days of treatment. Therefore, the rats were sacrificed after 40 days of treatment and the brains were collected at 0^0C.

Estimation of glutathione level

Tissues were homogenised in 10% TCA and after centrifugation the GSH content was measured in the supernatant using 5,5′-bisdithionitrobenzoic acid at 412 mμ[11].

Estimation of lipid peroxidation

Brain homogenates in phosphate buffer (0.1 *M* Na_2HPO_4 and KH_2PO_4; *p*H 6.7) were used to assay lipid peroxidation using thiobarbituric acid in presence of Fe^{2+} ion and the colour produced was read at 530 mμ[12].

Assay of enzymes

Superoxide dismutase was assayed following the method of Marklund and Marklund[13] by pyrogallol auotoxidation carried out in presence of 1 m*M* DTPA, 40 μg catalase, tissue homogenate in 0.1 *M* phosphate buffer of *p*H 8.5 in 2 ml. Freshly prepared 2.6 m*M* progallol solution(100 μl) in 10 m*M* HCl was added to attain a final concentration of 0.13 m*M*. Rate of change of absorbance at 420 mμ was taken for 2 min. Inhibition by 50% of autoxidation of pyrogallol was taken as one unit of SOD. No KCN was used to distinguish between Cu^{2+}-Zn^{2+} and Mn^{2+} SOD. Total SOD was taken in SOD activities.

Catalase was assayed using the method described by Chance and Machly[14] in which the decrease of light absorption at 240 mμ due to the decomposition of hydrogen peroxide by catalase was measured.

Glutathione peroxidase was assayed following the method of Günzler *et al.*[15] in which the oxidised glutathione is reduced by glutathione reductase followed by NADPH addition. The decrease in OD at 340 mμ is the index of NADPH utilisation for reduction.

Glutathione reductase was assayed by the method described by Brigelius *et al.*[16] in which glutathione disulphide is reduced by glutathione reductase, with NADPH as the coenzyme. NADPH itself is oxidised to NADP and decrease in OD at 340 mμ is the index of the glutathione reductase activity.

Protein was assayed following the method of Lowry *et al.*[17]

Results and Discussion

From Fig.1 it is seen that lipid peroxidaton in As treated rat brain increases significantly at all the doses tested, whereas, glutathione content decreases. The role of GSH in the detoxification of As has been reported to be through different mechanisms: (i) by facilitation of its uptake by the cell[18], (ii) by modulation of the first methylation reaction[19], (iii) by stimulation of the excretion of dimethyl arsenic acid[18], or (iv) may directly bind As. In the present study the level of GSH is decreased but is not correlated with increase in dose of As. The increase in lipid peroxidation in brain is not strictly dose related to As. Moreover, in cases of *in vitro* studies with the same doses the increase in lipid peroxidation and decrease in glutathione level show dose dependency upto 0.3 ppm concentration of As but at 3.0 ppm dose no such dose dependency is seen (Table 1).

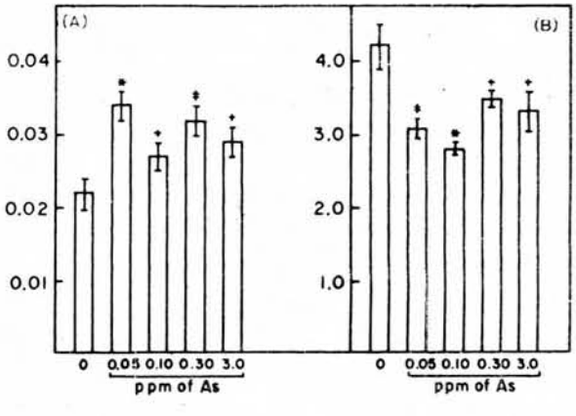

Fig.1—Effect of sodium arsenate containing drinking water on rat brain after forty days of treatment (A): Lipid peroxidation (ΔOD/mg protein); (B): glutathione (μg/mg) protein. [Results are expressed as mean ± SEM of 8 rats $^*p<0.001$; $^+p<0.05$; $^‡p<0.01$; $^§p<0.1$]

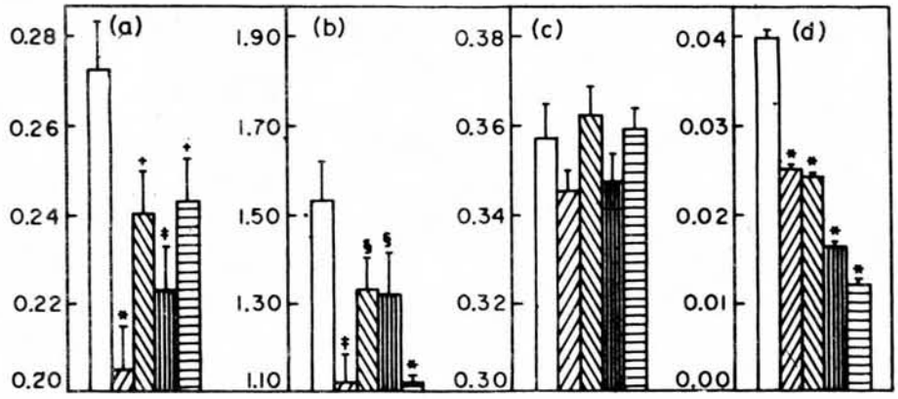

Fig.2—Activities of (a), superoxide dismutase; (b), catalase; (c), glutathione peroxidase; (d), glutathione reductase in rats given drinking water containing arsenic 0 ppm (□ control); 0.05 ppm (▨); 0.1 ppm (▩); 0.3 ppm (▦); 3.0 ppm (▤). [Results are expressed as mean ± SEM of 8 rats $*p<0.001$; $^{+}p<0.05$; $^{\ddagger}p<0.01$; $^{\S}p<0.1$]

Table 1—*In vitro* studies on rat brain lipid peroxidation (LPO) and glutathione (GSH) level in presence of arsenic [The values represents ±SEM of 8 rats]

Arsenic(ppm)	% LPO (ΔOD/mg protein)	% GSH (μg/mg protein)
Control (No As)	100±10	100±09
0.05	100±12	58±09
0.10	225±13*	35±10*
0.30	268±10*	35±12*
3.00	250±20*	141±11*

*$P<0.05$

Fig. 2 shows that superoxide dismutase in rat brain decreases with As treatment in all four groups, whereas, glutathione peroxidase activity remains unaffected. Glutathione reductase activity has decreased at all the four doses of As.

Glutathione peroxidase are found in high concentration in cell bodies of dopamine neurons[20]. Brain tissue contains low levels of GSH peroxidase and catalase but is rich in superoxide dismutase (both Cu^{2+}-Zn^{2+} and Mn^{2+} SOD)[21]. The reduction in SOD activity observed in brain in the present study indicates risk for brain cells.

Arsemic may induce lipid peroxidation in brain either by depletion of GSH or by inhibiting the enzymes of defence mechanism—superoxide dismutase, catalase and glutathione reductase or by generating higher than normal level of free radicals, even at the "permissible limit" of As in drinking water. The toxic effect may not be always dose dependent. A careful study of the "permissible limit" is warranted and safety of the potable water needs to be ensured.

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