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Arsenic Distribution and Neurochemical Effects in Peroral Sodium Arsenite Exposure of Rats

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Inorganic arsenic is ubiquitous in minerals (NATIONAL ACADEMY OF SCIENCES 1977) and sea water (BRAMAN & FOREBACK 1973). Inorganic and organic arsenicals are also used in technochemical products (WORLD HEALTH ORGANIZATION 1981). The exposed population may thus be large. The toxic effects of arsenic exposure include various clinical syndromes extending from neuropathy (MURPHY et al. 1981) to skin and liver malignancies (COWLISHAW et al. 1979) and teratogenic effects (WILLHITE 1981).

No single toxicological mechanism is known for these effects, and especially the origin of the neurological complications remains obscure (GOLDSTEIN et al. 1975). It is known that arsenic-induced neuropathy is related to the metalloid burden, and the condition regresses if arsenic is removed by chelation therapy (GOLDSTEIN et al. 1975; MURPHY et al. 1981).

While chemically related to phosphorus arsenic forms similar salts so that arsenate rapidly formed from As-trioxide in the mammals (YAMAUCHI et al. 1980) may replace phosphate e.g. in the nucleic acid metabolism and in production of ATP (MITCHELL et al. 1971). Therefore, the methylation of all arsenic compounds to methylarsonic and dimethylarsenic acids for their excretion is a significant detoxification reaction (YAMAUCHI & YAMAMURA 1979).

In this study, we exposed rats to sodium arsenite in drinking water at a moderate dose level to clarify the early effects on neural protein and energy metabolism. They were compared with the brain As burden and arsenic concentrations in other organs detected by a novel modification of existing analytical methods.

MATERIALS AND METHODS

Ten three-month-old male Wistar rats (313±35 g, ±S.D.) were given drinking water which contained 0.77 mM (0.1 g/l) sodium arsenite for 4 or 11 days. Ten similar rats (312±21 g) were maintained on tap water. The drinking rates were determined daily. The animals were killed by decapitation, and the left cerebral hemispheres, liver, kidneys, a blood sample taken in heparinized tubes (5 ml)

TABLE 1. Organ distribution of arsenic in peroral intake of sodium arsenite.

Days	Dose	Blood		Duodenum		Liver		Kidney		Brain	
		Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
4	138±29	67±6	1880±213	1.5±0.1	44± 8	3.9±0.4	61± 7	2.4±0.2	53± 8	0.9±0.06	24±3
11	403±20	67±4	3383± 72	1.5±0.3	58±10	4.7±0.4	116±21	3.1±0.2	161±37	1.0±0.1	63±9

Rats were given 0.77 mM sodium arsenite-containing drinking water for 4 or 11 days. The cumulative dose is given as μmol/kg body weight and the organ As concentrations as nmol/g (±S.D., N = 5).

and duodenum were analyzed for As as follows. Blood and lyophilized tissue samples (53.5-260 mg dry weight) were digested in 1 ml of a nitric, sulfuric and perchloric acid mixture (3:1:1, v/v/v). 100 µl of 65 % $Mg(NO_3)_2$ in water were added to each sample for a complete break-down of all As derivatives (LAUWERYS et al. 1979). 20 µl tributyl phosphate in each vial served as antifoam agent. The digestion was complete in 175 min while temperature increased gradually from 125°C to 275°C.

The reaction residue (0.2-0.3 ml) was washed out with 10 ml deionized water into the reaction vessel of a Hg hydride system (Perkin-Elmer 400, MHS-1). AsH_3 was formed at 950°C with sodium borohydride reduction by adding 2.5 ml 2.5 % NaOH and 5 % sodium borohydride mixture. The reaction vessels were purged with argon and the gas was conducted to a Perkin-Elmer atomic absorption spectometer equipped with arsenic electrodeless discharge lamp operated at 193.7 nm. Peak heights were used for the calculation of the As concentrations in the specimens.

The blanks, standards and reference standards (bovine liver, NBS Standard Reference Material 1577) were treated identically so that the signal to concentration ratio was shown to be practically independent of the original chemical nature of the As compound. Special care was taken to avoid contamination. To this end, all glassware was kept in detergent solution overnight, and was rinsed extensively after washing.

The detection limit of this method was 40 nmol As/l while the variation coefficient was 6.6 % at levels 50-100 µmol/l and 4.8 % at 1,700-3,500 µmol/l, respectively. The intraseries variation (N=6) was 5.3 % and the accuracy 84 % in comparison with the NBS standard (55 ng As/g).

Neuronal and glial cell fractions were isolated from the right cerebral hemispheres (SELLINGER et al. 1971) and an axonal fraction from the spinal cord specimens (SAVOLAINEN & VAINIO 1976). Acetylcholine esterase and succinate dehydrogenase activities (LIM & HSU 1971) were analyzed in neurons and in axons while creatine kinase, acid proteinase and 2',3'-cyclic nucleotide 3'-phosphohydrolase activities were analyzed in the glial cells (SAVOLAINEN et al. 1979). Statistical evaluation of the results was made with the Student's t-test.

RESULTS AND DISCUSSION

Control rats showed a steady As concentration in all tissues while exposed rats accumulated As according to the increasing dose (Table 1). The rate of accumulation was organ-specific with the highest rate in kidneys (Table 1) although blood As was the highest by a very broad margin at all times. It is notable that there was a virtual tissue-blood barrier in all organs. Duodenum did not accumulate As after 4 days (Table 1).

Virtually no neurochemical effects were seen at 4 days

while axonal acetylcholine esterase and succinate dehydrogenase activities decreased below the control level at 11 days (Table 2). The same enzyme activities remained comparable to controls in the neurons. Glial cell acid proteinase was above the controls at 11 days while the succinate dehydrogenase, creatine kinase and 2',3'-cyclic nucleotide 3'-phosphohydrolase remained unaffected (Table 2).

It is clear that inorganic arsenicals are absorbed in the gastrointestinal tract and there appears to be no uptake barrier in the duodenum as is the case e.g. for iron. The blood As in the control rats is rather high although it agrees with data in the literature (FUENTES et al. 1981; YAMAUCHI et al. 1980). In our case, it might have resulted from the commercial rat food arsenic

TABLE 2. Neurochemical effects of peroral sodium arsenite intake.

Neurons				Axons			
AChE		SDH		AChE		SDH	
Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
127±17	115±4	0.9±0.1	0.9±0.2	42±4	34±4**	2.4±0.1	2.1±0.1*
Glial cells							
CNP		SDH		CK		LAP	
Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
4.7±0.7	3.9±0.4	1.6±0.2	1.6±0.1	0.6±0.1	0.7±0.2	6.5±1.4	9.4±0.4**

Acetylcholine esterase (AChE), Succinate dehydrogenase (SDH), creatine kinase (CK) and lysosomal acid proteinase (LAP) activities are indicated as nmol/min x mg protein (N = 5, ± S.D.) and 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) as µmol/min x mg protein. Only data after the 11-day sodium arsenite exposure are presented. *$P < 0.05$, **$P < 0.01$.

content (7.5 nmol/g) while the special As affinity for hemoglobin might explain its high retention in blood (FUENTES et al., 1981).

Exposed rats remained similar to controls with similar weight gain (not shown) and similar water content in major organs (not shown). Therefore, the nervous system effects may reflect an early intoxication stage. The blood-brain barrier for As is comparable with barrier functions for other water-soluble contaminants (SAVOLAINEN 1982). The uptake is a function of glial cells (HIRANO & KOCHEN 1976). This should produce early glial cell effects. Marker enzyme for oligodendroglial cells, 2',3'-cyclic nucleotide 3'-phosphohydrolase (ROOTS 1981) and creatine kinase activity, marker enzyme for astroglial cells (THOMPSON et al. 1980) remained unaffected while the lysosomal acid proteinase activity increased above the controls. Depletion of ATP causes a loss of latency of lysosomal enzymes (RUTH & WEGLICKI 1982) so that impaired phosphorylation in glial cells could have explained this.

Impaired ATP metabolism could account for the biochemical effects in the spinal cord axons. Acetylcholine esterase is synthesized in the nerve cell bodies and transported by an energy-dependent mechanism to the more distal parts so that significant portion of axonal acetylcholine esterase activity results from enzyme en route to the axon terminals (COURAUD et al. 1982). Mitochondria also move along the axon by the energy-dependent tarnsfer (SAVOLAINEN 1982) so that the overall decrease in the axonal acetylcholine esterase and mitochondrial succinate dehydrogenase in the axons may be explained by the depletion of the ATP pool needed for the transport. It is also interesting to note that the early neuronal effects are confined to axons as the neuropathy is the characteristic finding in toxicity from arsenicals (GOLDSTEIN et al. 1975).

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