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Arsenic and oxidative stress in pentylenetetrazole-induced seizures in mice

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Abstract

Background and Objective: Chronic arsenic toxicity is a widespread problem; the role of brain oxidative stress has been suggested in the genesis of epilepsy and in the post-seizure neuronal death. However, studies investigating the effects of arsenic on seizure and related mechanisms are limited. The purpose of this study was to examine the effect of prolonged exposure to sodium arsenite on oxidative damage in pentylenetetrazole (PTZ)-induced seizures in mice.

Materials and Methods: In this study, male NMRI mice received sodium arsenite (0, 25, 50, and 100 ppm) in the drinking water for a period of 30 days. After exposure, all animals were injected PTZ (PTZ; 85 mg/kg, i.p.) to induce seizure, and the seizure parameters were evaluated for 30 minutes. Then, the levels of malondialdehyde (MDA) and reduced glutathione (GSH) were measured in the brain.

Results: The results of this study showed that sodium arsenite decreases the latency to the seizure onset and time of death ($p<0.05$). The greatest effect was observed at concentration of 50 ppm. The data indicated that exposure to sodium arsenite increases the levels of MDA ($p<0.05$) and decreased the levels of GSH in brain ($p<0.05$).

Conclusion: Our results suggest that PTZ effects potentiated by arsenic and oxidative damage involved in exacerbation of arsenic convulsive effects. Considering the role of arsenic in brain tissue damage following the seizure, it is recommended to control arsenic in drinking water.

Key words: Sodium arsenite, Oxidative damage, Seizure, Pentylenetetrazole

1. Introduction

Arsenic (As) is a toxic and hazardous metalloid. The term arsenic is from the Persian word Zarnikh, as interpreted to the Greek arsenikon, connotation "yelloworpiment." This metal has been known and used since antique times as the Poison of Kings and the King of Poisons (1). Arsenic is a public environmental and industrial contaminant worldwide. Environmental arsenic exposure mostly happens from arsenic-contaminated drinking water. Although greatest U.S. drinking water has arsenic at levels lower than 5 µg/L (ppb), it has been assessed that about 25 million people in Bangladesh alone drink water with arsenic levels above 50 ppb. Lots of people used up arsenic-contaminated drinking water in the countries of Mexico, Argentina, China, Bangladesh, India and Iran (2-8).

Chronic arsenic exposure can outcome in multisystem sicknesses. Many papers have

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demonstrated that arsenic exposure leads to neurological injuries (9-14). However, studies investigating the effects of arsenic on seizure and related mechanisms are limited. Arsenic and its metabolites have been shown to create oxidative damage, alteration in DNA methylation status and genomic instability. A number of studies in recent years have focused on the role of oxidative stress in seizures (15-19). Experimental seizures are known to be associated with a massive release of reactive oxygen species. An increase in lipid peroxidation has been reported in the brain of rodents following PTZ-induced seizures and kindling (20-23).

Pentylenetetrazole (PTZ), an epilepsy inducing agent, is a selective blocker of GABAA receptor-chloride ionophore complex, which induces convulsions by triggering the glutamatergic transmitter system. This activation is due to an increased intracellular calcium ion influx, which results in an increased production of superoxide radicals (23-26). Considering the high concentrations of arsenic in drinking water and its effects in brain tissue damage, the purpose of this study was to examine the effect of prolonged exposure to sodium arsenite on oxidative damage in PTZ-induced seizures

2. Materials and Methods

2.1. Chemicals

Sodium arsenite (99% pure), pentylenetetrazole, thiobarbituric acid, trichloroacetic acid, and reduced glutathione were purchased from Sigma-Aldrich (St Louis, Missouri, USA), and 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), 1,1,3,3-tetramethoxypropane was obtained from Merck (Darmstadt, Germany).

2.2. Exposure protocol

40 male NMRI mice (30-35 g) were obtained from the animal facility of Ahvaz Jundishapur University of Medical Science (AJUMS), which is fully accredited by AJUMS animal care guidelines with an ethics committee approval No.IR.AJUMS.REC.1396.254. The study was conducted from September to November 2017 at School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The mice were grouped and housed in polycarbonate cages under controlled conditions of 25±2°C temperature with a 12h light/ 12h dark cycle and 10% humidity. The previous studies showed that mice might be less susceptible than human to arsenic toxicity, partly due to a faster metabolism and clearance of arsenic. Therefore, it is necessary to use higher exposure concentration of arsenic than the environmentally relevant concentrations in mouse experiment. Indeed, a recent report showed that it took 10 times higher concentration of drinking water arsenic (50 ppm) to achieve arsenic concentrations similar to those seen in human exposed to arsenic in west Bengal (27). Mice were divided into four groups (n=10): 0 ppm (control group), As 25 ppm, As 50 ppm, and As 100 ppm. Therefore, mice drank diH_2O or diH_2O plus arsenite in doses of 25, 50, and 100 ppm for a period of 30 days in the present study. Water containing arsenite was freshly prepared every three days to minimize its oxidation.

2.3. Seizure induction

After arsenic exposure duration, PTZ was dissolved in sterile isotonic saline and administered intraperitoneally at a dose of 85 mg/kg. The animals were observed for a period of 30 min and the latency to first convulsion, the number of seizure episodes, the occurrence of hind limb tonic extensions (HLTE), the time to death and mortality were recorded.

2.4. Oxidant/Antioxidant analysis

Whole brain malondialdehyde (MDA) and reduced glutathione (GSH) content were estimated. GSH, an indicator of antioxidant levels, was estimated in the brain suspension by the DTNB reagent. The developed yellow color was spectrophotometrically read at 412 nm (UV-1601PC; Shimadzu, Japan). The MDA content was determined in terms of thiobarbituric acid reactive substance (TBARS) formation. Since 99% of TBARS was MDA, TBARS concentrations of the samples were calculated from a standard curve using 1, 1, 3, 3-tetramethoxypropane.

2.5. Statistical analysis

All data were statistically analyzed using Graph Pad Prism (version 5.04) as mean ± standard error of mean (SEM) with one-way analysis of variance (ANOVA), followed by post hoc Tukey test. Moreover, differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Seizure activity analysis

Exposure to sodium arsenite did not significantly affect the number of HLTE ($p>0.05$) but the significant change in the number of seizures and number of deaths were seen ($p<0.05$). The latent period between PTZ induction and seizure and time to death are decreased in the PTZ + arsenic groups compared with the convulsive control group mice ($p<0.05$) (Figures 1 and 2). Comparison of time of death in control (PTZ), As (25 ppm) + PTZ, As (50 ppm) + PTZ, and As (100 ppm) + PTZ groups showed that arsenic at concentrations of 50 and 100 ppm significantly reduces the time of death ($p<0.05$), however the difference between 50 and 100 ppm was not significant ($p>0.05$) (Figure 1). Latencies to

generalized seizures onsets in control (PTZ), As (25 ppm) + PTZ, As (50 ppm) + PTZ, and As (100 ppm) + PTZ groups showed that arsenic concentrations of 50 and 100 ppm significantly reduces the time for onset of seizure ($p<0.05$), however in this case it was not statistically significant between 50 and 100 ppm ($p>0.05$) (Figure 2) (Table 1). Interestingly in opposite of what was expected, reduction of the number of seizures was observed with increasing dose of arsenic ($p<0.05$). Comparison of the number of seizures shows that arsenic at concentrations of 50 and 100 ppm (especially in concentration of 100 ppm) significantly reduced of the number of seizures ($p<0.05$).

Table 1. Effect of As exposure on average time of seizures onset, time of death, number of seizure, HLTE and mortality.

As intake (ppm)	Time of seizures onset (minute)	Time of Death (minute)	Number of seizure	Number of HLTE	% mortality
0	2.030±0.2909	24.08 ±3.503	1.848 ±0.2897	2.000±0.5774	50%
25	0.8950 ± 0.2378*	15.80 ±5.834	0.9960 ±0.3849	2.000±0.5774	75%
50	0.6225 ± 0.1275**	6.198 ±1.711*	0.7040±0.2046***	1.000±0.0	100%
100	0.7200 ±0.2232**	7.363 ±1.527*	0.4222 ±0.1888**	1.000±0.0	100%

Data are given as Mean±SEM of different experiments.*: Significantly different from control $p < 0.05$, ** $p< 0.01$ and *** $p< 0.001$.

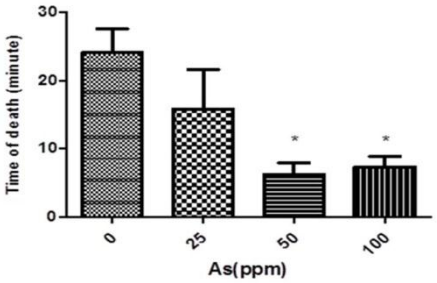

Figure 1. Comparison of time to death in control (PTZ), As (25 ppm) + PTZ, As (50 ppm) + PTZ, and As (100 ppm) + PTZ groups. The animals were treated with distilled water or sodium arsenite (25, 50 or 100 ppm) through drinking water for 1 month before a single injection of PTZ (85 mg/kg). * $p < 0.05$ when compared with control.

3.2. Oxidant/Antioxidant analysis

The results of lipid peroxidation revealed that malondialdehyde level was significantly higher in arsenic treated groups (especially: 100 ppm) compared with the control groups mice ($p<0.05$). Glutathione assessment results showed a significant decrease in the PTZ + arsenic groups compared with the control group mice ($p<0.05$). In addition, comparison of the MDA and GSH levels in brain tissues showed that MDA level in the brain increases with increasing concentrations of arsenic and at a concentration of 100 reached its highest level and is notably significant ($p<0.05$). And vice versa in the case of glutathione with increasing levels of arsenic, GSH level decreases. This decrease is evident at doses of 50 and 100 ppm ($p<0.05$) (Figures 3 and4). Results demonstrated that arsenic treatment increases MDA, oxidative stress indicators, and reduces GSH level in brain tissue after PTZ-induced seizure.

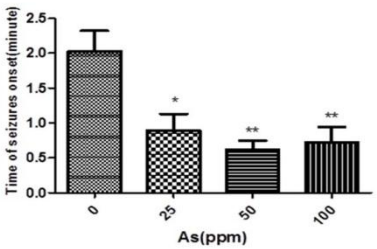

Figure 2. Latencies to generalized seizures onsets in control (PTZ), As (25 ppm) + PTZ, As (50 ppm) + PTZ, and As (100 ppm) + PTZ groups. The animals were treated with distilled water or sodium arsenite (25, 50 or 100 ppm) through drinking water for 1 month before a single injection of PTZ (85 mg/kg). * $p < 0.05$ and ** $p < 0.01$ when compared with control.

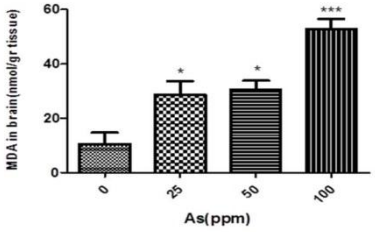

Figure 3. Comparison of the MDA levels in brain tissues of control (PTZ), As (25 ppm) + PTZ, As (50 ppm) + PTZ, and As (100 ppm) + PTZ groups. The animals were treated with distilled water or sodium arsenite (25, 50 or 100 ppm) through drinking water for 1 month before a single injection of PTZ (85 mg/kg). * $p < 0.05$ and *** $p < 0.001$ when compared with control.

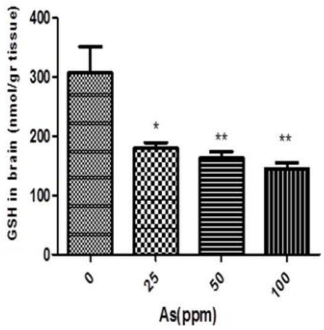

Figure 4. Comparison of the GSH levels in brain tissues of control (PTZ), As (25 ppm) + PTZ, As (50 ppm) + PTZ, and As (100 ppm) + PTZ groups. The animals were treated with distilled water or sodium arsenite (25, 50 or 100 ppm) through drinking water for 1 month before a single injection of PTZ (85 mg/kg). * $P < 0.05$ and ** $p < 0.01$ when compared with control.

4. Discussion

Arsenic is a naturally occurring metalloid worldwide. Human chronic exposure to inorganic arsenic (iAs), which are at the top of hazardous substances, is related with different diseases containing cancer and non-cancerous diseases. The neurotoxic effects of iAs and its methylated metabolites have been established in exposed people and experimental models (14, 28-30). In this study for the first time, we have demonstrated that arsenic elevates oxidative brain damage after PTZ-induced seizure. New studies propose that oxidative stress is important in brain tissue damage following seizure stimulation (17, 31, 32). An improved generation of ROS, i.e., hydroxyl radicals as well as products of lipid peroxidation developments in cerebral tissue of rats with PTZ-evoked seizures has been described (21, 33).

Multiple mechanisms have been suggested to contribute in arsenic-induced toxicity. Besides its recognized capacity to induce oxidative stress, arsenic also interacts with cellular targets such as the thiol groups of various proteins. In fact, glutathione (GSH) is required at several stages for metabolic conversion of both arsenite [As (III)] and arsenate [As (V)]. It has been suggested that arsenite constitutes a bypass for electrons from the respiratory chain, thereby facilitating the formation of superoxide anion radical (29, 34, 35). Additional suggested mechanisms include the reduction of oxygen by As(III), thereby leading directly to the generation of H_2O_2 and/or development of peroxyl radicals as central mediators of DNA destruction(36). It remains hard to clarify the individual significance of each of the potential toxic mechanisms of arsenic, although there is increasing consensus regarding a principal role for ROS generation (37).

Our results demonstrated that the As treatment increases MDA, the oxidative stress indicators, and reduces GSH level in brain tissue after PTZ-induced seizure. A reduction in the GSH levels might outcome in the reduced elimination of superoxide ion and hydrogen peroxide radicals, which carries about a sum of reactions that are destructive to the neuronal tissue (38). The results of this study showed that sodium arsenite decreased the latency to the onset of seizures and time of death. The greatest effect was observed at concentrations of 50 ppm. Considering the high concentrations of arsenic in drinking water and its effects in brain tissue damage behind seizure and the treatment of seizure, contamination of drinking water by arsenic should be investigated or controlled. In our study, interestingly number of seizures reduced with increasing dose of arsenic and this outcome was opposite to what was expected which may have been related to increased mortality in high arsenic concentration treated mice and should be considered in future studies. Similar to many other toxicants, neurobehavioral alterations after arsenic exposure is affected by several intervening variables (among which are the concentration and duration of exposure, along with experimental conditions), so different concentrations of As may act through different pathways and produce different behavioral results (39-41).

The main limitation of this study was the lack of studies assessing prolonged exposure effects of arsenic and the constant focus on acute severe arsenic poisoning in seizures and epileptic seizures. The precise mechanisms by which these perturbations happen have not been determined and should be considered in future studies.

In conclusion, our data support a role for arsenic via oxidative stress in neuronal damage after seizure attacks and it is recommended to control arsenic in drinking water.

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Conflict of Interest: None declared.

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