Inorganic arsenic alters the development of dopaminergic neurons but not serotonergic neurons and induces motor neuron development via Sonic hedgehog pathway in zebrafish

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Abstract

The mechanism of inorganic arsenic-induced neurotoxicity at the cellular level is not known. In zebrafish, teratological effects of inorganic arsenic have been shown at various concentrations. Here, we used similar concentrations of inorganic arsenic to evaluate the effects on specific neuron types. Exposure of zebrafish embryos at 5 hours post fertilization (hpf) to sodium arsenite induced developmental toxicity (reduced body length) in 72 hpf larvae, beginning at a concentration of 300 mg/L concentration. Mortality or overt morphological deformity was detected at 500 mg/L sodium arsenite. While 200 mg/L sodium arsenite induced development of tyrosine hydroxylase-positive (dopaminergic) neurons, there was no significant effect on the development of 5-hydroxytryptamine (serotonergic) neurons. Sodium arsenite reduced acetylcholinesterase activity. In the *hb9-GFP* transgenic larvae, both 200 and 400 mg/L sodium arsenite produced supernumerary motor neurons in the spinal cord. Inhibition of the Sonic hedgehog (Shh) pathway that is essential for motor neuron development, by Gant61, prevented sodium arsenite-induced supernumerary motor neuron development. Inductively coupled plasma mass spectrometry (ICP-MS) revealed that with 200 mg/L and 400 mg/L sodium arsenite treatment, each larva had an average of 387.8 pg and 847.5 pg arsenic, respectively. The data show for the first time that inorganic arsenic alters the development of dopaminergic and motor neurons in the zebrafish larvae and the latter occurs through the Shh pathway. These results may help understand why arsenic-exposed populations suffer from psychiatric disorders and motor neuron disease and Shh may, potentially, serve as a plasma biomarker of arsenic toxicity.

Introduction

Arsenic is an element with metalloid characteristics. Exposure to arsenic is known to be toxic to living organisms including humans. Human beings are exposed to arsenic through inhalation, water, food, agricultural and industrial products, and drugs. Arsenic is neurotoxic as it is capable of crossing the blood-brain barrier (BBB) and accumulating in the brain. Several studies suggest that inorganic arsenic exposure at early life can adversely affect central nervous system (CNS) function. A delay and inhibition of neurodevelopmental process of the CNS during the fetal and early life stages in humans exposed to inorganic arsenic have also been reported. Arsenic can cause neural and behavioral changes in rats, and decrease axonal acetylcholinesterase activity in the rat spinal neurons (reviewed in Kanungo et al., 2023)

Arsenic exposure through drinking water at concentrations of 10–50 ppb cause peripheral neuropathy in humans. In children, impairment of the CNS may occur at ≥50 ppb; however, in adults, only high concentrations of inorganic arsenic are known to cause CNS impairment. Further, a prior report indicated an increased risk (16.7%) of mortality due to motor neuron disease (MND) in a population exposed to inorganic arsenic. MND is a neurodegenerative disease in which motor neuron functions decline progressively in the CNS (reviewed in Kanungo et al., 2023).

Zebrafish embryos are an ideal model system to study the effects of drugs and chemicals on several organs including the nervous system. In zebrafish embryos/larvae, 500 ppb inorganic arsenic in water altered neurobehavior. The effects of inorganic arsenic on various neuron types in a developing vertebrate at the cellular level are not clear. The present study was undertaken to determine the effects of inorganic arsenic (sodium arsenite) on overall development and the nervous system (motor neurons, dopaminergic neurons, and serotonergic neurons) using both wild type and transgenic zebrafish embryos/larvae.

Materials and Methods

Both wild type (WT) and *hb9-GFP* transgenic embryos were used for this study. For treatment with sodium arsenite (100 – 500 mg/L), 5 hpf embryos were used. This dose range was chosen based on a report that there was no phenotypic change in vascular development or gross morphology at 10 mg/L sodium arsenite, but at 400 mg/L (3.08 mM). For each treatment, embryos were placed in one of the wells of 6-well plates containing 5 ml embryo medium. Embryo medium contains 15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, and 0.7 mM $NaHCO_3$. At specific experimental time points, WT embryos or larvae were collected for assessment of morphological deformity, heart rate measurement, body length measurement, apoptosis detection, and immunohistochemical analyses. *Hb9-GFP* transgenic larvae were processed for imaging (motor neurons) in vivo (Kanungo et al., 2023).

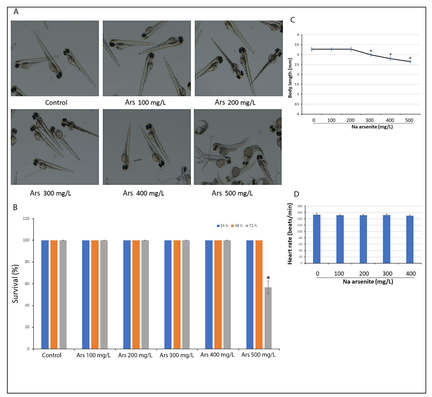


Figure 1. Effect of sodium arsenite on the early development of zebrafish and survival. Embryos at 5 hpf were exposed (static) to 100, 200, 300, 400 or 500 mg/L of sodium arsenite. After 68 h of exposure (larva age – 72 hpf), (A) larval development was monitored and images were acquired. (B) Mortality rate (C) Body length, and (D) heart rate were measured. Asterisk indicates statistical significance.

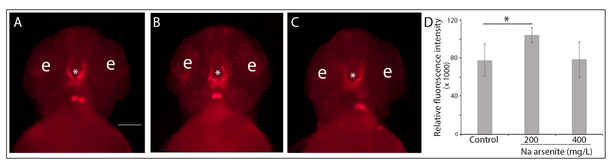


Figure 2. Effect of sodium arsenite on tyrosine hydroxylase-positive (TH-positive) dopaminergic neurons during early development in zebrafish. Embryos at 5 hpf were exposed (static) to 200 or 400 mg/L of sodium arsenite. Whole-mount immunohistochemistry was performed to identify TH-positive neurons in the brain. Images show the ventral side of the brain. (A) Control; (B) 200 mg/L arsenic exposure; and (C) 400 mg/L arsenic exposure. (D) Relative fluorescence intensity of the TH-positive neurons in the control and 200 or 400 mg/L sodium arsenite-treated larvae is shown. Asterisk indicates statistical significance.

	pg/larva, average ± sd, n=4				
Treatment Group	^{55}Mn	^{65}Cu	^{66}Zn	^{75}As	
Control	57.1 ± 11.2	199.9 ± 19.9[a]	8,379 ± 1,325	23.8 ± 2.4	1.07×10^6 pg/g body weight
400 mg/L $NaAsO_2$	51.1 ± 9.4	172.1 ± 21.9	7,658 ± 360	847.5 ± 73.4[b,c]	2.51×10^6 pg/g body weight

Levels of ^{55}Mn, ^{65}Cu, ^{66}Zn, and ^{75}As in zebrafish larvae treated with 0, 100, 200, or 400 mg/L NaAsO2. ([a]n = 3, [b]Significantly different ($p < 0.05$) from control larvae, [c]Significantly different ($P < 0.05$) from larvae treated with 100 or 200 mg/L NaAsO2).

Results and Discussion

Our data show for the first time that sodium arsenite alters neurogenesis of dopaminergic neurons and motor neurons, which may explain motor neuron disease and neuropsychiatric disorders in populations exposed to arsenic. Importantly, our study shows for the first time that Shh signaling is involved in sodium arsenite-induced motor neuron development. This study suggests that Shh, a secretory protein, can potentially be a plasma biomarker of arsenic exposure.

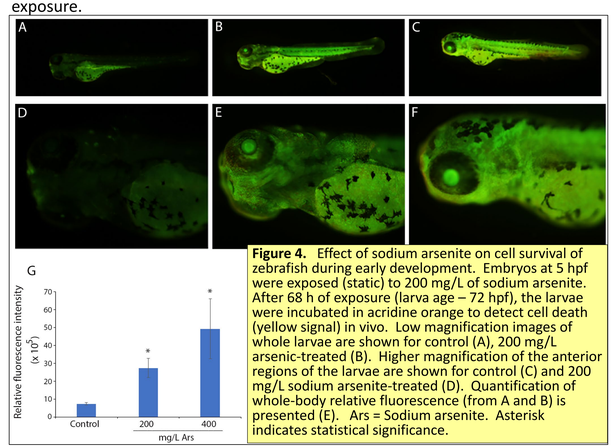


Figure 4. Effect of sodium arsenite on cell survival of zebrafish during early development. Embryos at 5 hpf were exposed (static) to 200 mg/L of sodium arsenite. After 68 h of exposure (larva age – 72 hpf), the larvae were incubated in acridine orange to detect cell death (yellow signal) in vivo. Low magnification images of whole larvae are shown for control (A), 200 mg/L arsenic-treated (B). Higher magnification of the anterior regions of the larvae are shown for control (C) and 200 mg/L sodium arsenite-treated (D). Quantification of whole-body relative fluorescence (from A and B) is presented (E). Ars = Sodium arsenite. Asterisk indicates statistical significance.

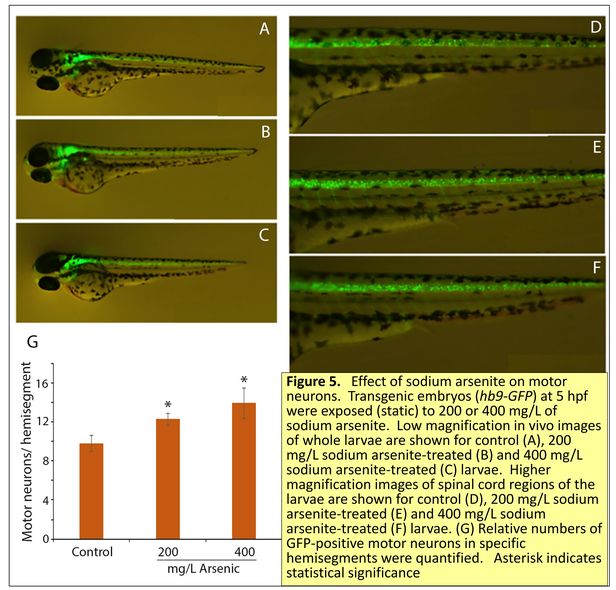
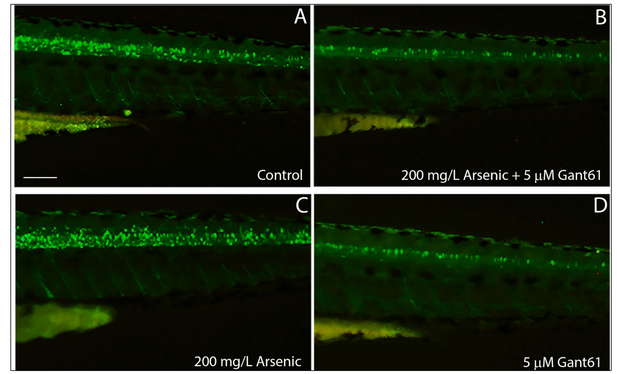


Figure 5. Effect of sodium arsenite on motor neurons. Transgenic embryos (*hb9-GFP*) at 5 hpf were exposed (static) to 200 or 400 mg/L of sodium arsenite. Low magnification in vivo images of whole larvae are shown for control (A), 200 mg/L sodium arsenite-treated (B) and 400 mg/L sodium arsenite-treated (C) larvae. Higher magnification images of spinal cord regions of the larvae are shown for control (D), 200 mg/L sodium arsenite-treated (E) and 400 mg/L sodium arsenite-treated (F) larvae. (G) Relative numbers of GFP-positive motor neurons in specific hemisegments were quantified. Asterisk indicates statistical significance



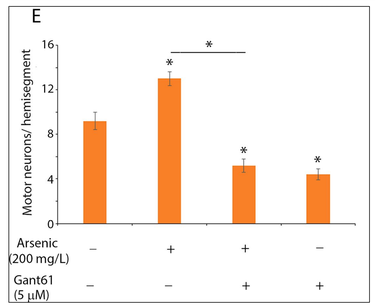


Figure 6. Effect of Shh inhibitor, Gant61, on arsenic-induced supernumerary motor neuron development. Transgenic embryos (hb9-GFP) at 5 hpf were exposed (static) to 200 mg/L of sodium arsenite. Fluorescent images of spinal cord regions of the larvae are shown for control (A), 200 mg/L sodium arsenite-treated (B), 200 mg/L sodium arsenite with 5 μM Gant61 treated (C), and 5 μM Gant61-treated larvae. (E) Relative numbers of GFP-positive motor neurons in specific hemisegments were quantified

Conclusion

1. Sodium arsenite significantly reduced acetylcholinesterase activity.
2. Sodium arsenite increased dopaminergic neurons, but not 5-HT (serotonergic) neurons in the brain.
3. In *hb9-GFP* transgenic zebrafish, sodium arsenite induced generation of supernumerary motor neurons in the spinal cord potentially through Shh signaling pathway.
4. Anomalous motor neuron development may explain why arsenic exposure causes motor neuron disease/death and Shh could be a potential plasma biomarker of arsenic toxicity

Reference

Kanungo J., Twaddle, N., Silva, C., Robinson, B., Wolle, M., Conklin, S., MacMahon, S., Gu, Q., Edhlund, I., Benjamin, L., Beland, F., Fitzpatrick, S. (2023) Inorganic arsenic alters the development of dopaminergic neurons but not serotonergic neurons and induces motor neuron development via Sonic hedgehog pathway inzebrafish. Neurosci Lett., 2023;795:137042