The effects of Arsenite on learning and memory Caenorhabditis elegans

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ABSTRACT

The nematode Caenorhabditis elegans has emerged as an important animal model in various fields including neurobiology, developmental biology, and genetics. Characteristics of this animal model that have contributed to its success include its genetic manipulability, invariant and fully described developmental program, well-characterized genome, ease of maintenance, short and prolific life cycle, and small body size. These same features have led to an increasing use of C. elegans in toxicology, both for mechanistic studies and high-throughput screening approaches. We describe some of the research that has been carried out in the areas of neurotoxicology, genetic toxicology, and environmental toxicology, as well as high-throughput experiments with C. elegans including genome-wide screening for molecular targets of toxicity and rapid toxicity assessment for new chemicals. We argue for an increased role for C. elegans in complementing other model systems in toxicological research. Years before the latest technologic developments (RNAi and high-throughput techniques), C. elegans was used to study toxicity mechanisms of environmental factors affecting the nervous system. The following section provides a synopsis of the available literature on neurotoxicity-related issues addressed in C. elegans. It is not meant to be exhaustive but rather to illustrate typical studies that are amenable in the C. elegans platform. We highlight studies with exposure outcomes to various metals and pesticides, as well as general considerations on studies of neurodegenerative diseases. We emphasize the utility of C. elegans in addressing hypothesis-driven mechanisms of neurotoxicity and extrapolations to vertebrate systems.

Keyword: - *C. elegans1, Neurotoxic Metals2, Caenorhabditis elegans3, and Arsenic Toxicity4*

1. INTRODUCTION

Caenorhabditis elegans is a small transparent, unsegment Nematode that lives in temperate soils. C.elegans is a multicellular organism which goes through a complex growth starting as an embryo, undergoing a morphogenesis and developing into a fully formed adult (life cycle 2 to 3 days). It exhibits specific behaviours and is even capable of rudimentary learning and memory. It produces eggs, mates and reproduces. After reproduction it gradually ages, loses vigor, and finally dies. C.elegans is a model organism for the study learning and memory, physical behaviour and aging. These organisms easily grow and maintained in the laboratory. C.elegans is excellent model organism to the heavy metal. Therefore, the C.elegans model system is valuable for investigation of arsenite toxicity. C.elegans makes a good model for the study of many biological processes. The worms are easy and inexpensive to maintain on solid media in petri dish. They have a life span of 2-3 weeks and progress from egg to fully develop into gravy worms. Arsenic is metalloid, which is distributed throughout the earth crust in diverse complex forms with pyrites. Depending on the physiochemical condition of the environment, arsenic can readily be dissociated from the complex, enter into ground water and taken up by microorganism resulting in high level of bio-availability. The high concentration of arsenic in water and soil can be found in several places around the world. Two different oxidative states of arsenic III and V, are available in organic and inorganic forms that correlate with their cytotoxic potential. Between these two states, compound with (III) oxidation state are more toxic to target cells and tissue due to several mechanism including high affinity for protein thiol or vicinal sulfhydryl group. Although the metabolism of inorganic arsenic is quite well known, the precise mechanism of arsenic toxicity is not clearly

understood. In mammals, a methylation pathway has been proposed for the metabolic processing of inorganic arsenicals. Other aspects of arsenic metabolism in C.elegans remain to be seen. In this thesis report, I will be treated arsenite in synchronized L1 stage larvae with different concentration (0.125mg/lit, 0.25mg/lit, 0.5mg/lit). After the treatment of arsenite in 24 hours, observe the sample. The results show, arsenite is lethal for living being.

2. LITERATURE REVIEW

Learning and memory are fundamental biological properties that appeared to be acquired in the early era of animal evolution. Because of simple neuronal circuits and easy access to experiments, invertebrates have played an important role in understanding the biological basis of learning and memory. Studies on Aplysia and Hermissenda revealed the essential mechanism of synaptic plasticity [1,2]. Similarly, the behavioral molecular genetics in Drosophila and Caenorhabditis elegans greatly advanced our knowledge on the nervous system [3–5]. Invertebrate studies found neural logic commonly used throughout evolution. Much of neurotransmitters and neuronal modulators used in C.eleganssuch as acetylcholine, glutamate, dopamine, serotonin, GABA, and neuropeptides are amazingly similar to those used in mammals [8]. Genome project revealed that genes required for neuronal development and function are also highly homologous to mammalian genes [9,10]. Rapidly advanced technologies such as calcium imaging and optogenetics are particularly accessible to the C.elegans nervous system, thereby enabling the worm researchers to extensively study dynamics of neurons and circuits. These advantages of C.elegans allow comprehensive and high-resolution studies for understanding human brain and neuronal disorders. C.elegans apparently exhibits behaviors that reflect learning and memory [11–19].

2.1 Life Cycle of C.elegans 1

Life cycles of C.elegans has short and temperature dependent. Its life span is around 2 to 3 weeks under suitable living condition. C.elegans adults lay embryo that pass through gastrulation, comma stage, 2- fold and 3-fold embryos before hatching in the L1 larval form. The larvae develop through L2, L3, and L4 molts before becoming adults. When the food or other source becomes scarce, worms enter into diapause stage.

Dauer larvae were first identified as a special larval stage of insect-parasitic nematodes. These larvae, which differed structurally from all other stages of the same species, were termed “dauer larven” by Fuchs (1915). The dauer (enduring) stage of Caenorhabditis elegans is formed when environmental conditions are inadequate for successful reproduction. In abundant food, the animal develops continuously through the four larval stages (L1–L4) to the adult. Dauer larvae do not feed, but they can survive at least four to eight times the normal 2-week life span of C.elegans (Klass and Hirsh 1976). When favorable conditions are encountered, the dauer larva begins to feed and resumes development to the adult. Dauer larvae are easily distinguished from other developmental stages. They are thin and dense due to shrinkage of the hypodermis at the dauer-specific molt. The dauer larva exhibits a metabolism that is consistent with long-term survival in the absence of food. Dauer larvae have reduced TCA cycle activity but high phosphofructokinase activity relative to adults, indicating that dauer larvae have a greater capacity to metabolize glycogen (O'Riordan and Burnell 1989). The decreased TCA cycle activity relative to the glyoxylate cycle in dauer larvae indicates the importance of lipid storage as an energy reserve in the dauer stage.

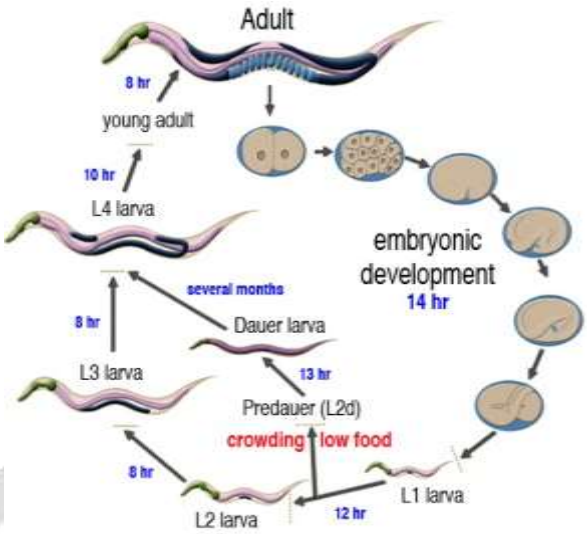


Figure 1 - life cycle of C.elegans and potential Diapauses(Taken from Nanette Pazdenik and Tim Schedl, 2013.

2.2 Arsenic Toxicity

Arsenic is a metalloid, which present in earth crust in the form of pyrites. It is found in air, water and soil and could find their way into the human body through: Inhalation, Ingestion and Absorption. Continuous arsenic exposure via drinking water has been reported in many countries of the world; especially in Argentina, Bangladesh, India, Mexico, Thailand, and Taiwan, where a large proportion of ground water is contaminated with a high concentration of arsenic. Arsenic contaminated air, water and food is associated with reproductive and developmental diseases. Also, it causes diseases including cancer, dermal, cardiovascular, hepatic, renal, peripheral vasculature maladies, dysfunction of the endocrine, bladder and kidney [1,2]. The nervous system is highly vulnerable to the toxic effects of arsenic, resulting into multiple neurological effects [3]. The epidemiological studies indicate that behavioural and cognitive functions in children are compromised following gestational and developmental arsenic exposure. Owing to increased exposure associated with food intake patterns and lifestyle of individuals impose higher risk to nervous system [4]. The developing brain is vulnerable to toxic metals that interfere with the critical developmental processes i.e., cell proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis in the central nervous system (CNS) [19]. Arsenic toxicity-induced defects in nervous system includes Peripheral Neuropathy, Alterations in peripheral nerves, Neuritism retrobulbar neuritis; neuropathy, Encephalopathy, Abnormal EEG's, numbness in extremities; parathesia, Abdominal Pain, Depression, mood swings, flat affect, impaired facial recognition, Mental retardation, borderline intelligence, Hearing loss and difficulty hearing, Decreases locomotor activitiy, Convulsions; seizure, Muscle pain; headache and acrodynia [20].

Two different oxidative states of arsenic III and V are available in organic and inorganic forms that correlate with their cytotoxic potential. Between these two states, compound with (III) oxidation state are more toxic to target cells and tissue due to several mechanism including high affinity for protein thiol or vicinal sulfhydryl group. Although the metabolism of inorganic arsenic is quite well known, the precise mechanism of arsenic toxicity is not clearly understood. In mammals, a methylation pathway has been proposed for the metabolic processing of inorganic arsenicals. Other aspect of arsenic metabolism in C.elegans remain to be seen.

3. MATERIALS AND METHODS

3.1 Preparation of Arsenic solution

We prepared stock solution and respective amount of waves was added to prepare the following concentration of arsenic solution. Arsenic solution was prepared by 0.125mg of arsenic dissolves in 1 liter water. Arsenic solution was prepared by 0.25mg of arsenic dissolve in 1 liter of water.

Arsenic solution was prepared by 0.5mg of arsenic dissolved in 1 litre of water.

4.3. Methodology

Prepared LB media

↓

Inoculated OP50 E.coli strain into LB media

↓

Incubated it at 37°C for over night

↓

Prepared NGM plates

↓

Inoculated bacterial culture into NGM plates

↓

Incubated NGM Plates at 37°C for over night

↓

Transferred C.elegans on OP50 inoculated NGM Plates

↓

Kept it into the incubator at 17°C

↓

When C.elegans grew into gravid (egg-laying) worms, synchronized the plates to get L1 stage larvae

↓

Treated L1 stage larvae with different concentration of arsenic (0.75mg/L, 1.5 mg/L, 3.00mg/L, and 6.00mg/L) and incubated it at 17°C for 24 hrs.

↓

Counting of live and dead L1 larvae to find the mortality rate.

For daf-2 mutant of C.elegans, we used the same protocol mentioned above to find the mortality rate.

Table 1: Chemical

S.No.	Chemicals	Company	Country
1	NaCl	Merck	India
2	Hypochlorite	……..	……..
3	LB Media	Himedia	India
4	Agar	Himedia	India
5	Peptone	Himedia	India
6	CaCl2	Merck	India
7	Cholesterol	Himedia	India
8	Ethanol	Changshu Yangyuan Chemical	China
9	MgSO4	Fisher Scientific	India
10	KPO4		
11	NaOH	Merck	India
12	KOH	Merck	India
13	KH2PO4	Merck	India
14	K2HPO4	Merck	India
15	Na2HPO4	Merck	India
17	Na2AS2O4	Himedia	India

Table 2: Preparation of LB media for 1 liter

S.No.	Chemical	Amount
1.	Tryptone	10 gm
2.	Yeast extract	5 gm
3.	NaCl	10 gm

4.	Distilled water	1000 ml

Table 3: Preparation of Nematode Growth Media (NGM) For 1 liter

S.No.	Chemicals	Amount
1.	NaCl	3.0 gm
2.	Peptone	2.5gm
3.	Agar	18.0 gm
4.	Distilled Water	Maintained vol. to 900 ml

Table 3 Autoclaved it and at the time of pouring we added the following chemicals and maintained the volume to 1 liter:

S.No.	Chemicals	Amount
1.	CaCl2	1 ml
2.	Cholesterol in ethanol	1 ml
3.	MgSO4 (1M)	1 ml
4.	KPO4 (1M) (pH-6)	25 ml

Table 3 Buffer required in synchronization Hypochlorite buffer: For 10 ml

S.No.	Chemicals	Amount
1.	Hypo	4 ml
2.	NaOH (1M)	2 ml
3.	KOH (1M)	4ml

Table 3 M9 buffer: For 1 Liter:

S.No.	Chemicals	Amount
1.	KH2PO4	3 gm
2.	Na2HPO4	6 gm
3.	NaCl	5 gm
4.	MgSO4(1M)	1 ml
5.	d H2O	

Maintained its volume with distilled water after mixing all chemicals or after preparation of buffer and autoclaved it and store it at 4°C.

3.2. Preparation of C.elegans bacterial food source

The most favourite food of C.elegans is OP50 strain of E.Coli and it is usually grown monoxencally in the laboratory. E.coli OP50 is a uracil auxotroph whose growth is limited on NGM plates. This helps in getting a limited bacterial lawn which is desirable for easier observation and better mating of the worms. We obtained the culture of E.coli OP50 by inoculating LB media with starter culture and incubating them at 37°C.

3.3. Preparation of NGM Petri plates

We prepared NGM petri plates by pouring Nematode Growth Medium aseptically into petri plates. On this plate, C.elegans is maintained in laboratory. This NGM plates can be left at room temperature for 2-3 days before use to allow excess moisture to evaporate. Plates stored in an air-tight container at room temperature will be usable for several weeks.

3.4. Seeding NGM Plates

We applied 100 µl E.coli OP50 liquid culture to NGM plates in the laminar hood. If desired, the drop of liquid culture can be spread using the pipet tip or a glass rod. Spreading will create a larger lawn, which can aid in

visualising the worms. Now, allow the E.coli OP50 lawn to grow overnight at 37°C. OP50 seeded NGM plates stored in an air-tight container will remain usable for 2-3 weeks.

3.5. Transferring worms on OP-50 seeded NGM plates

Several methods are used for transferring C.elegans from one petri plates to another. A quick and convenient method is Chunking, wherein a sterilized scalpel or spatula is used to move a chunk of agar from an old plate to a fresh NGM plate. There will be usually 100 of worms in the chunk of the agar. The worms will crawl out of the chunk and spread out onto the bacterial lawn of new NGM plate. This method works well for transferring worms that have burrowed into the agar or are difficult to pick individually. Another method of transferring is to pick single worm with a worm picker. A worm picker can be made by mounting a 1inch piece of 32-gauge platinum wire into either the tip of a pasture pipet or in a bacteriological loop holder. Now, the chunked petri plates were at 17◦C in incubator for the proper growth of C.elegans.

3.6. Synchronization

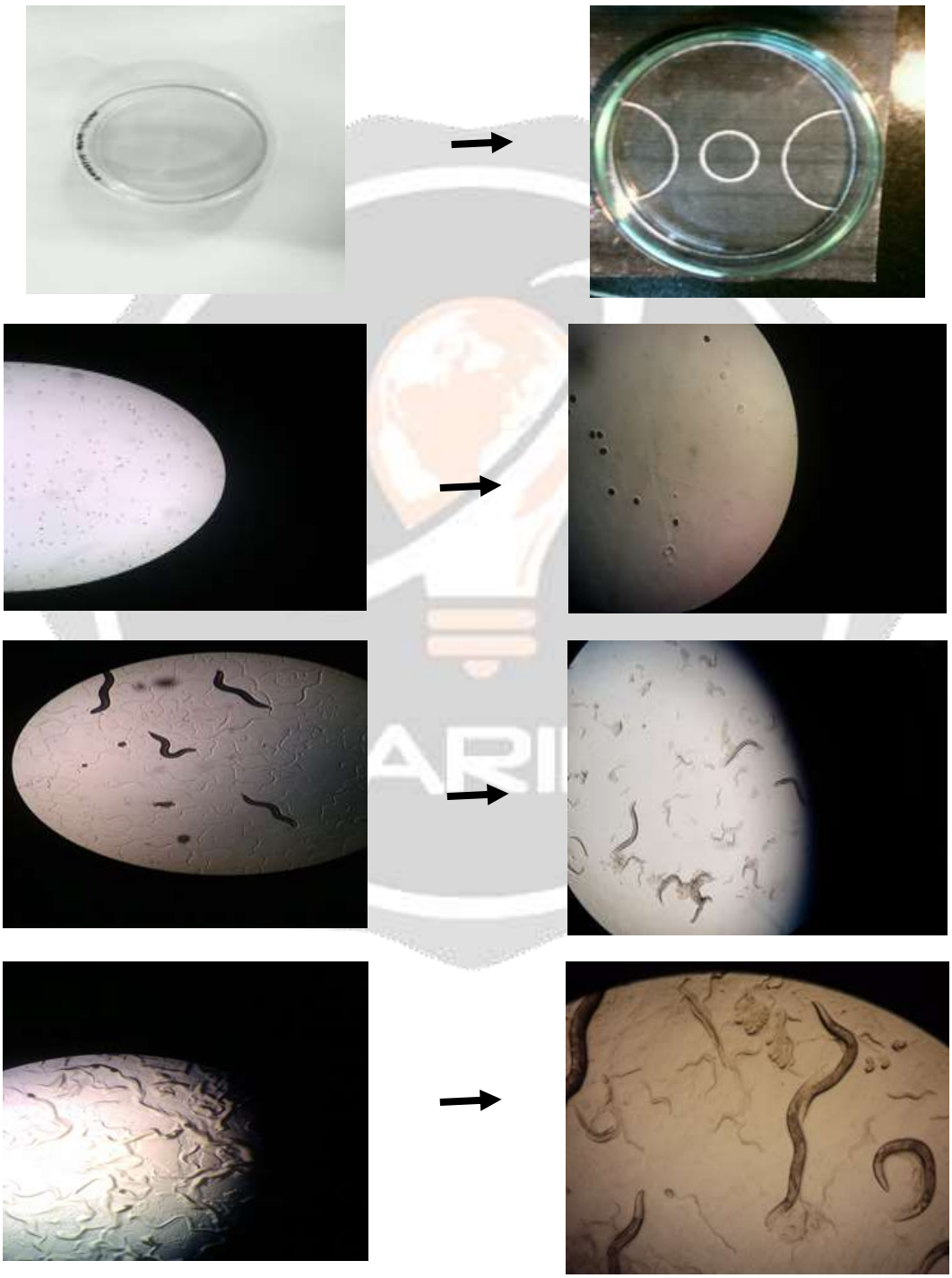
Synchronization is the process in which all gravid C.elegans were arrested in L1 stage. C.elegans were bleached with hypochlorite to collect eggs which were incubated at 17◦C for overnight to bring them to synchronized L1 stage.

The protocol of synchronization is mentioned below: -

Pour distilled water into the NGM plate containing adult or mature C.elegans
↓
Centrifuge it at 3000 rpm for 2 minutes
↓
Discard supernatant
↓
Add hypochlorite solution about 4 ml
↓
Vortex/Shake it for 4-5 minutes
↓
Centrifuge it at 2000 rpm for 2 minutes
↓
Discard supernatant
↓
Pellet dissolved in distilled water 203 minutes
↓
Centrifuge it at 2000 rpm for 2 minutes
↓
Add M9 buffer about 3-4 ml and dissolve pellet
↓
Centrifuge it at 2000 rpm for 2 minutes
↓
Again wash it with distilled water 2 times
↓
Centrifuge it at 2000 rpm for 2 minutes
↓
Discard supernatant
↓
Dissolved pellet in about 2-3 ml of distilled water
↓
Pour it into the sterile petriplate
↓
Keep it at 16°/17°C in incubator

4. RESULTS AND DISCUSSIONS

These are the different pictures of C.elegans which were taken by us in our laboratory. Our data suggest that the percentage of dead L1 Worms increase with the increase in arsenic in arsenic Concentration. At the different concentration of 0.125mg/l, 0.25mg/l and 0.5mg/l, all the L1 stage were Statically data recorded.



4. CONCLUSIONS

Arsenic is a common environmental metalloid widely distributed around the world and its exposure causes several types of health problem including cancer in human. Epidemiologic evidence of neurotoxicity associated with arsenite (ASIII) exposure from different source via water source. It has been reported that arsenic is toxic to Hippocampus.

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