

The Effect of Anatoxin-a on Mice's Body Systems During FHABs

Shanshan Huang

Abstract

Currently, freshwater resources are a threat by the freshwater harmful algal blooms(FHABs) and human impact. Among the effects of FHABs, the harmful caused by cyanobacteria. The cyanobacteria are very obvious because they can produce cyanotoxin (dermatotoxins, hepatotoxins, and neurotoxins) and anatoxin-a. It is most likely to cause death in humans and animals. Also, anatoxin-a has been implicated in animal mortality and can cause death in minutes. As a result, it is essential to research anatoxin-a, one of the neurotoxins, from three distinct aspects: mode of action and chronic or sublethal effects..

Keywords: anatoxin-a, freshwater harmful algae bloom(FHABs), CD-1 mice, acetylcholine receptors, In Vivo Screen, righting reflex, negative geotaxis time.

1. Introduction

Nowadays, excess reproduction and accumulation of cyanobacteria can result in cyanobacterial blooms in aquatic environments. Cyanotoxin, toxic metabolites produced from cyanobacteria, are a global concern (Chorus and Bartram, 1999) because it may adversely bring acute and chronic effects to both humans and animals. Based on Rutkowska et al., (2019), among three main groups of cyanotoxins (dermatotoxins, hepatotoxins, and neurotoxins), anatoxin-a is one of the neurotoxins, results in the most likely underlying cause of death. In July 2002, several healthy teenage boys became ill with mild to severe symptoms after swimming in a blue-green alga scum-covered golf course pond in Dane County, Wisconsin (Chelsea A. Weirich, BS, and Toss R. Miller, Ph.D., 2014). After 48 hours, one boy even died of heart failure. However, the bloom and stool samples tests indicated that the death was not correlated to certain pesticides, parasites, and other pathogens because the results were negative. After years of research, researchers have found that anatoxin-a's occurrence was the most likely underlying cause. At the same time, several concerns about freshwater harmful algal blooms (FHABs), which cause the color change to blue-green on the water's surface, have attracted public attention. Cyanobacteria, sometimes called blue-green algae, are organisms that cause freshwater harmful algae bloom (FHABs). In Freshwater Harmful Algal Blooms: Toxins and Children's Health, researchers have found that FHAB toxins may harm humans in recreational environments surged when the incidence of FHABs increases.

This study will research anatoxin-a, one of the neurotoxins, from three distinct aspects: mode of action, chronic or sublethal effects. In contemporary society, the conservation of water resources is the primary duty of human beings. FHABs, cyanobacteria, and neurotoxins, including anatoxin-a, put pressure on drinking water

safety. As a result, it is necessary for humanity to understand thoroughly the protracted influence brought by cyanotoxins, for example, anatoxin-a. Therefore, this article will concentrate on the effects of anatoxin-a on mice's body systems during FHABs to explore the possible impact on humankind's body systems so the solutions can be considered.

As a result, the social concerns raised above beg an essential research question: To answer the research question: what are anatoxin-a's chronic or sublethal effects on mice's body systems during FHABs that may also affect humans? The hypothesis for the above research question can be formulated briefly according to the most inspiring preceding article, Freshwater Harmful Algal Blooms: Toxins and Children's Health (Chelsea A. Weirich, BS, and Toss R. Miller, Ph.D., 2014). Cyanobacteria are the primary producers of freshwater harmful algal blooms, and anatoxin-a is one of the neurotoxins made by cyanobacteria. This study predicts that anatoxin-a damages nerves and muscle tissue and binds acetylcholine receptors at the synapses between nerves and muscle tissues, altering action potentials and leading to overstimulation of muscle leading to fatigue. So in the next part of this study, treat mice with increasing amounts of anatoxin-a for various durations and measure neurological testing (negative geotaxis measure, righting reflex). Mice will be monitored for death, clinical signs of intoxication, and neurological testing. Groups of pregnant mice will be injected with a different amount but the same concentration of anatoxin-a solution.

This study is essential to the field that researches neurotoxins, algal blooms, and FHABs, particularly anatoxin-a. Also, the study will help more people who are interested in environmental toxins, the nervous system, and some aspects of environmental protection and encourage them to pay close attention to water resources protection and put forward more relevant solutions to save

more lives from FHABs and neurotoxins influencing.

2. Materials and methods

Some methods, including liquid chromatography (LC), ultraviolet-visible spectrophotometry (UV-Vis), and enzyme-linked immunosorbent assays (ELISAs), rely on time-consuming chemical extraction and need high-end equipment. As a result, the control-variable method and time-pregnant mice will be used to get the proper results and conclusions in this study. Mice will be treated with increasing amounts of anatoxin-a for various durations, and dose-finding data will be measured inside the mice's body by comparing with the clinical signs of toxicity, modification of the teratology screen, and neurological testing (negative geotaxis measure, righting reflex). As the introduction mentioned, mice will be monitored for death and clinical signs of intoxication (decreased motor activity, rough hair coat, altered gait, convulsions). All the following information and design are based on the Potential developmental toxicity of anatoxin-a, a cyanobacterial toxin written by *E. H. Rogers, E. S. Hunter III, V. C. Moser, P. M. Phillips, J. Herkovits, L. Muñoz, L. L. Hall and N. Chernoff* in 2005.

2.1 materials needed in the experiments

CD-1® IGS mice are collected from the Charles River Laboratories. All the mice will be housed in plastic cages with optimum temperature change between 22 to 26 Celsius and 12 hours light cycle to maintain a stable environment. The anatoxin-a, which has a purity \geq of 90%, will be injected in three different doses(0, 125, 200 $\mu\text{g kg}^{-1}$). The pregnant mice are used because the behavior of pregnant mice and their pups can be both detected, so the results will be more comprehensive.

2.2 experiments design

This part of the experiment will be carried out at least three times to ensure the credibility of the results. In the

experiments, the pups were divided into two groups: one includes the weight measuring in In Vivo screen testing and another is the neurological testing in order to measure the righting reflex times and negative geotaxis time. Also, doing a titration treatment is necessary to prepare the solution with proper concentration. At the same time, the pregnant number, dead number, and treated number are also collected. All data will be tabulated and displayed in conclusions.

2.3 Expected results

2.3.1 In Vivo Screen testing

When the range of gestation days keeps constant, as the amount of anatoxin-a injection in mice's bodies increases, the number of living pups will reduce because of the lethal effect brought by concentrated anatoxin-a solution. However, the different pregnancy conditions, which means there are a different number of mice under a natural conception, will slightly affect the results. Still, the proportion can be taken to analyze.

2.3.2 In neurological testing

I predict that the anatoxin-a can bind to the acetylcholine receptors and cause an unable righting reflex. To be more specific, the animal first returns the head to its normal position, which is caused by the feeling of being lost, and the reflex center is in the midbrain. However, when the injection amount of concentrated anatoxin-a solution increases, the trunk will remain still in an incorrect position, the cervical muscles will be twisted, and the stimulation of the muscle spindles are going to send out a second reflex (central distribution in the midbrain or thoracic spinal cord) that restores the trunk to the proper position.

3. Possible results

This part is the prediction of results in the experiments, and all the results contain uncertainty to a certain extent. Some of the results are based on the previous study.

Injection with an increasing amount of anatoxin-a (purity \geq of 90%, in three different doses(0, 125, 200 $\mu\text{g kg}^{-1}$))					
		Possible result (1)	Possible result (2)	Possible result (3)	Possible result (4)
In Vivo screen testing		○	△	▽	●
Neurological testing	Righting reflex time	+	--	□	▲
	Negative geotaxis time	*	+	--	□
△ the bodyweight of pups increases ○ the bodyweight of pups unchanged. + the time taken increases -- the time taken decreases * unserviceable result					
▽ the bodyweight of pups decreases ● sudden death □ unchanged ▲ mice are unable to finish					

Figure 1. the possible results that may be collected from the experiments with different amount but the same concentration of anatoxin-a injected.

4. Discussion

4.1 possible results analysis in In Vivo screen testing

According to *Comparison of effects of anatoxin-a(s) and paraoxon, physostigmine, and pyridostigmine on mouse brain cholinesterase activity (1988)* and *Potential developmental toxicity of anatoxin-a, a cyanobacterial toxin (2005)*. The level of anatoxin-a postnatal toxicity in the mouse can be shown as motor activity decreased immediately after treatment in pregnant dams injected with anatoxin-a at a concentration of 200 µg.

There will have four possible results: (1) This result contradicts the hypothesis. There may not be an effect on pup weight or viability on either PND 1 or PND 6 in mice treated on GD 8–12; (2) This result can contradict the hypothesis entirely. There will be an increase in pup weight because the anatoxin-a may stimulate cell growth to an extent; (3) The result fully supports the hypothesis. The body weight of pups may be decreased slightly with increasing the amount of anatoxin-a injected because of the possible inhibiting effects; (4) Partially supporting result for the hypothesis. Sudden death may also exist because the injected amount of anatoxin-a may achieve a lethal dose.

4.2 possible results analysis in neurological testing

4.2.1 In righting reflex time test

There also have four possible results: (1) Fully supporting result for the hypothesis. When the amount of injected anatoxin-a increases, the righting reflex time of female mice becomes longer in PND6, PND12, and PND20, which can also be analyzed as slower muscle contraction, which means there will be a decrease in the number of acetylcholine receptors between synapses in muscles; (2) Contradicting result for the hypothesis. The anatoxin-a may have the ability to stimulate the transduction of neurotransmitters (for example, acetylcholine) or to increase the number of acetylcholinesterase (AChE) on the surface membrane of the postsynaptic neuron. As a result, the righting reflex time of female mice will be shorter; (3) Contradicting result for the hypothesis. There will be no change in the time taken for righting reflex time because there will be no effect of injecting an increasing amount of anatoxin-a; (4) Partially supporting result for the hypothesis. When the amount of anatoxin-a injected reaches a lethal dose (different in each individual), the mice will be unable to finish the righting reflex.

4.2.2 In negative geotaxis time

Four possible negative geotaxis times may exist (1) Unserviceable result for the hypothesis. The negative geotaxis time cannot be collected because the infant rodents placed on inclined surfaces (ranging from 15 degrees to 70 degrees in most tests) are posturally unstable, and their compensatory responses have been misinterpreted as negative geotaxis; (2) Fully supporting result for the hypothesis. The negative geotaxis time will increase as the injected amount of anatoxin-a increases. As the prediction mentioned, the increasing injected amount of anatoxin-a will influence the muscle function and even cause fatigue, so the time taken for negative geotaxis will be longer than before; (3) Contradicting result for the hypothesis. There will have a decrease in the negative geotaxis time. So, the anatoxin-a will not speed up the muscle contraction; (4) Contradicting result for the hypothesis. The negative geotaxis time will remain unchanged.

4.3 Evaluations

4.3.1 Expectancy

Neurotoxins, algal blooms, and FHABs, particularly anatoxin-a, all require this research. Furthermore, the study will help more people who are interested in environmental toxins, the nervous system, and some aspects of environmental protection by encouraging them to pay close attention to water resource protection and propose more relevant solutions to save more lives from the effects of FHABs and neurotoxins. Simultaneously, this paper focused on the impacts of anatoxin-a on mice's body systems during FHABs in order to investigate the potential influence on human body systems and provide remedies.

4.3.2 Limitations and ethical considerations

The neurological test will have unintended consequences, such as pups falling off the screen before turning in one dosing group. If the pups died after sliding off, it might have altered the trial findings. Because the study employed pregnant CD-1 mice as samples, there are few ethical concerns to be made.

4.3.3 Improvements and advice

In Vivo Screening, testing and neurological testing were employed in the investigations, which are both extracorporeal assessments. More endosomatic measures, such as BMAA or UV-Vis, can be done in the future to acquire accurate findings.

5. Conclusions

To answer the research question: what are anatoxin-a's chronic or sublethal effects on mice's body systems during

FHABs that may also affect humans?

All the conclusions are made based on the possible results collected from the experiments with pregnant CD-1 mice. As the possible results are shown above in the diagram, the research question can be answered. Anatoxin-a may damage nerves and muscle tissue and binds to acetylcholine receptors at the synapses between nerves and muscle tissues, causing overstimulation of muscle leading to fatigue because the times needed for pregnant female mice to finish righting reflex are longer. Also, when the injected amount of anatoxin-a reaches the lethal dose, there will be fatal consequences.

This research is critical to neurotoxins, algal blooms, and FHABs, especially anatoxin-a. In addition, the study will assist more people interested in environmental toxins, the nervous system, and some aspects of environmental protection by encouraging them to pay close attention to water resource protection and propose more relevant solutions to save more lives from the effects of FHABs and neurotoxins.

In the future, humankind should pay more attention to protecting water resources. At the same time, we should take more measures to inhibit the FHABs and cyanotoxin release. Also, I believe that the adverse effects brought by FHABs and anatoxin-a will be cut down in the coming future.

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