MMSL Military Medical Science Letters

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2011, vol. 80, p. 129-139
ISSN 0372-7025
DOI: 10.31482/mmsl.2011.019

REVIEW ARTICLE

ANATOXIN-A(S): NATURAL ORGANOPHOSPHORUS ANTICHOLINESTERASE AGENT

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Received 1th July 2011.
Revised 22th August 2011.
Published 9th September 2011.

Summary

Anatoxin-a(s) is a guanidinemethyl phosphate ester isolated from the freshwater cyanobacterium (blue-green algae) *Anabaena flos-aquae* strain NRC 525–17. Previous work has shown anatoxin-a(s) to be a potent irreversible inhibitor of electric eel acetylcholinesterase (AChE, EC 3.1.1.7). Anatoxin-a(s) has been shown to be an active site-directed inhibitor of AChE, which is resistant to reactivation by oximes because of the enzyme-oxime adduct formation. *In vivo* pretreatment with physostigmine and high concentrations of pyridine 2-aldoxime methochloride (2-PAM) were the only effective antagonists against a lethal dose of anatoxin-a(s). Anatoxin-a(s) is very toxic and it is produced by cyanobacteria during its blooms. Purified toxin has an LD_{50} (i.p) of approximately 20-50 μg/kg body weight in mice. Toxicoses associated with cholinesterase-inhibiting anatoxin-a(s) have been observed in humans, animals, birds and fish. Anatoxin-a(s) induces clinical signs of hypercholinergic preponderance, such as salivation, lacrimation, urinary incontinence, defecation, convulsion, fasciculation, and respiratory arrest.

Key words: natural organophosphate; anatoxin-a(s); anticholinesterase; chemistry; pharmacology; toxicology; blue-green algae; neurotoxin

INTRODUCTION

Acetylcholinesterase inhibiting (anti-ChE) organophosphorus compounds(OP) are a group of chemicals such as nerve agents and pesticides that are synthesized and used for various purposes, including human and veterinary medicine (Bajgar, 2004; Gupta, 2006, 2009; Satoh and Gupta, 2010). OPs are denoted with a general formula (Figure 1), proposed by Gerhard Schrader in 1937. Basically, these OPs with insecticidal properties are esters of phosphoric acid (Figure 1, Schrader, 1950). This implies that a biologically active OP compound contains an oxygen or sulphur, two similar or dissimilar side chains

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bound to the phosphorus atom, and an organic or inorganic acid residue (leaving group, x):

R^1 and R^2 are capable of almost infinite variation. They may represent alcohols, phenols, thiols, amides, alkyl or aryl groups attached directly to phosphorus. A common X radical may be halogene, cyanide, thiocyanate, etc. An OP compound becomes a strong AChE inhibitor and consequently more toxic when S is replaced by O.

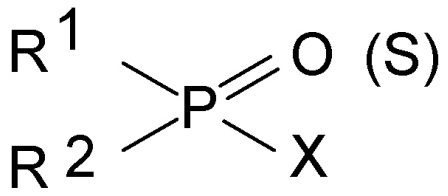

Figure 1. Common Structure of Organophosporous Compounds

Anatoxin a(s) – A natural organophosphate

Cyanobacteria, formerly called "blue-green algae", are simple and primitive photosynthetic microorganisms, which widely occur in fresh, brackish and salt waters. George Francis first reported a toxigenic bloom in Nature in 1878, a "poisonous Australian lake" with "a thick scum like green oil paint" and vividly described acute intoxications of sheep, horses, dogs, and pigs (Puschner and Roegner, in press). Analysis of archaeological evidence coupled with an evolving understanding of modern blooms have begun to implicate the role of cyanotoxin poisoning to more widespread mammalian die-offs dating back to the Pleistocene age, i.e. about 150,000 years BC (Braun and Pfeiffer, 2002). A controversial hypothesis about the role of cyanobacteria in the various mass extinction events has begun to emerge (Castle and Rodgers, 2009). By now, 40 different genera and 2000 species of cyanobacteria are known and 80 species of them are known to produce potent toxins responsible for a wide array of human illnesses, aquatic mammal and bird morbidity and mortality, and extensive fish kills. These cyanotoxins act as neurotoxins or hepatotoxins and are structurally and functionally diverse, and many are derived from unique biosynthetic pathways (Patocka, 2001; van Apeldoorn et al., 2007; Aráoz et al., 2010; Humbert, 2009: Puschner and Roegner, 2012).

Anatoxins are mainly produced by cyanobacteria in the *Anabaena* genus (Beltran and Neilan, 2000), but also by other genera, such as *Plantkothrix*, *Oscillatoria*, *Microcystis*, *Aphanizomenon*, *Cylindorspermum*, and *Phormidium* (Sivonen and Jones, 1999). Anatoxins are neurotoxins and can be divided into anatoxin-a, homoanatoxin-a, and anatoxin-a(s). Anatoxin-a(s) is a natural OP, which has a unique *N*-hydroxyguanidine methylphosphate ester with a chemical structure (CAS Registry No. 103170-78-1,(5S)-2-amino-1-((hydroxy-methoxypho-sphinyl) oxy)-N,N-dimethyl-4,5-dih ydro-1H-imida-zole-5-methanamin; Mol Wt., 252 daltons) as shown in Figure 2. Anatoxin-a(s) has been commonly detected in the United States (Monserrat et al., 2001) and Europe (Henriksen et al., 1997). Anatoxin-a(s) acts as a non-competitive potent irreversible inhibitor of AChE activity, preventing it from hydrolyzing the neurotransmitter acetylcholine (Mahmood and Carmichael, 1986). The letter "s" corresponds to the characteristic symptom of salivation caused in vertebrates, mainly in doses close to LD_{50} levels of 20-40 µg/kg (Matsunaga et al., 1989; Falconer, 1998). As a consequence, acetylcholine (ACh) remains available to bind membrane receptors, resulting in continuous muscle stimulation. When respiratory muscles are also affected, death may ensue by respiratory failure and brain hypoxia (Matsunaga et al., 1989, Carmichael and Falconer, 1993).

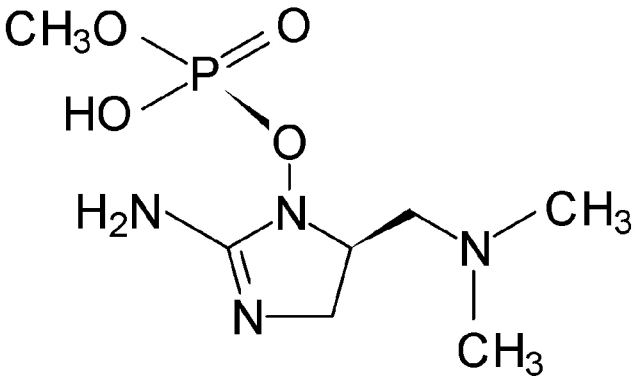

Figure 2. Chemical structure of anatoxin-a(s).

Isolation

Anatoxin-a(s) is produced by *Anabaena flos-aquae* clone NRC 525-17 and can be purified from lyophilized cells. Purification procedure involved extraction with 1.0 M acetic acid: ethanol (80:20), column chromatography (Sephadex G-15 and CM-Sephadex C-25) and high performance liquid chromatography. Purified toxin has a repored LD_{50} (i.p) of approximately 20-50 µg/kg body weight in mice (Mahmood and Carmichael, 1986; Falconer, 1998).

Synthesis and Biosynthesis

The alkyl chain of anatoxin-a(s) (cyclic guanidines), which can be used as an intermediate in the total synthesis of anatoxin-a(s), was synthesized recently in both racemic and enantiomerically pure forms (Moura and Pinto, 2010). C-2, C-4, C-5 and C-6 of the anatoxin-a(s) are derived from the guanido carbon, C-5, C-4 and C-3 of L-arginine, respectively, and the three *O,N*-methyl groups originate from the tetrahydrofolate C_1 pool. *Erythro*-4-Hydroxy-l-arginine, a minor constituent of the cyanophyte, may be a precursor in the biosynthesis (Moore et al., 1992).

Anticholinesterase properties

The *in vitro* inhibition of electric eel acetylcholinesterase (AChE, E.C. 3.1.1.7) and horse serum butyrylcholinesterase (BuChE, E.C. 3.1.1.8) by anatoxin-a(s) was time- and concentration-dependent. The inhibition of electric eel AChE follows first order kinetics, indicative of irreversible inhibition. The irreversibility of electric eel AChE inhibition was confirmed by a plot of V_{max} versus total enzyme concentration (Mahmood and Carmichael, 1987).

The kinetics of inhibition of ChE by anatoxin-a(s) supports the theory that signs of intoxication are primarily due to ChE inhibition. Assays of serum ChE of rats in an acute toxicity study showed a complete inactivation of the enzyme at doses of 350 and 600 µg anatoxin-a(s)/kg body weight. It was concluded that anatoxin-a(s) may be acting as an anti-ChE, thereby causing toxicity (Mahmood and Carmichael, 1987). Cook et al. (1988) compared anatoxin-a(s) to paraoxon, physostigmine and pyridostigmine for effects on brain ChE after i.p. injection into mice. The duration of clinical signs in mice injected with anatoxin-a(s) was comparable to that of paraoxon and sign persisted longer than with carbamates. Anatoxin-a(s) did not inhibit brain ChE activity, suggesting that this toxin is unable to cross the blood-brain barrier. Experiments of Cook and his co-workers (1989a) validated that anatoxin-a(s) is strictly a peripheral ChE inhibitor. When rats were injected (i.p) with 1.5, 3.0 or 9.0 µg/kg of anatoxin-a(s) or with 800 µg/kg of paraoxon, unlike paraoxon, anatoxin-a(s) did not cause detectable inhibition of ChE in the central nervous system, but did cause inhibition of ChE in blood.

Mechanism of action

Hyde and Carmichael (1991) investigated the interaction of anatoxin-a(s) with AChE and

suggested that this natural OP is an irreversible active-site directed inhibitor of AChE. Some similarities have been noted between anatoxin-a(s) and the synthetic anti-ChE OP. The reversibility of cholinesterase inhibition in plasma, red blood cells, and diaphragm in mice given anatoxin-a(s) was characterized by Cook and his co-workers (1991) and was compared with the effects of two known ChE inhibitors, the OP compound paraoxon and the carbamate compound pyridostigmine bromide. The time required for recovery from anatoxin-a(s)-induced inhibition of ChE in plasma, red blood cells, and diaphragm was similar to or longer than that with paraoxon and longer than that with pyridostigmine. Based on the duration of anatoxin-a(s) induced clinical signs and ChE inhibition in mice, anatoxin-a(s) appears to be an *in vivo* irreversible inhibitor of ChEs (Cook et al., 1991). In another study, Cook et al. (1989c) demonstrated that anatoxin-a(s) inhibits AChE activity only in the periphery, while the OP or carbamate can inhibit AChE in the brain and retina as well.

An intoxication by anatoxin-a(s) is characterized by cholinergic syndromes similar to OP insecticide and nerve agent intoxications. Anticholinesterase neurotoxins, as well as other toxins, have some disadvantages if used as weapons of mass destruction. For these reasons, it should be necessary to control these neurotoxins through international treaties which have real verification measures, such as the Chemical Weapons Convention (Pita et al., 2003).

Toxicokinetics

Toxicokinetic data of anatoxin-a, homoanatoxin-a and anatoxin-a(s) have not yet been reported. But the rapid onset of clinical signs after oral exposure indicated rapid absorption of the toxin from the gastrointestinal tract. In a recent study, Puschner et al. (2010) detected anatoxin-a in urine and bile of a poisoned dog, revealing that anatoxin-a, at least in part, is excreted unchanged in urine and bile.

Pharmacology and toxicology

Pharmacological tests of anatoxin-a(s) on isolated chick (*biventer cervicis*) and frog (*rectus abdominis*) muscles showed no direct agonistic effect. Instead, anatoxin-a(s) augmented the acetylcholine response and antagonized the actions of *d*-tubocurarine. Twitch potentiation and tetanic fade were observed on isolated rat phrenic nerve-diaphragm muscle when stimulated indirectly at different frequencies (Mahmood and Carmichael, 1986). Anatoxin-a(s), given intraperitoneally (i.p) to Sprague-Dawley rats at different doses (0.1-1.0 mg/kg body weight), caused signs of severe cholinergic overstimulation (Mahmood and Carmichael, 1987). Based on i.p LD_{50} in mice, anatoxin-a(s) is reported to be 10 - 12-fold more toxic than anatoxin-a and homoanatoxin-a (Stevens and Krieger, 1991; Skulberg et al., 1992; Briand et al., 2003). In acute toxicity tests in mice and rats, the signs of poisoning were indicative of excessive cholinergic stimulation. Mice pretreated with atropine sulfate showed longer survival times and no muscarinic signs of toxicity. The mice still died of respiratory arrest with convulsions, which indicated that toxicity is due to more than just the peripheral muscarinic action of anatoxin-a(s) (Mahmood and Carmichael, 1986).

The effects of anatoxin-a(s) on mean arterial blood pressure, heart rate, respiratory rate, tidal volume, minute volume, and phrenic nerve activity were evaluated in anesthetized Sprague-Dawley rats by Cook et al. (1989b). The initial effect of the anatoxin-a(s) was to slow the heart rate and reduce arterial blood pressure, followed by much more pronounced reductions in these parameters. The marked decline in heart rate and blood pressure frequently occurred before there was a large decrease in respiratory minute volume, suggesting that anatoxin-a(s) has an important muscarinic effect on the cardiovascular system *in vivo*. Phrenic nerve amplitude increased, but, nevertheless, tidal and minute volumes decreased progressively, indicating that anatoxin a(s), unlike most low-molecular-weight OP cholinesterase inhibitors, does not have any remarkable inhibitory action on central mediation of respiration (Cook et al., 1989b).

Ecotoxicology

Cyanobacteria are found in lakes, ponds, rivers and brackish waters throughout the world. In cases of excessive growth, such as bloom formation, these bacteria can produce inherent toxins in quantities causing toxicity in mammals, including humans (van Apeldoorn et al., 2007).

Worldwide development of cyanobacterial blooms has significantly increased in marine and continental waters in the last century due to water eutrophication. This phenomenon is favoured by the ability of planktonic cyanobacteria to synthesize gas vesicles that allow them to float in the water column. Benthic cyanobacteria that proliferate at the bottom of lakes, rivers and costal waters form dense mats near the shore. Cyanobacterial massive proliferation is of public concern with regards to the capacity of certain cyanobacterial strains to produce hepatotoxic and neurotoxic compounds that can affect the health of wild animals, livestock and humans (Aráoz et al., 2010; Puschner and Roegner, 2012). Ecological effects of cyanobacterial toxins are also rapidly expanding in the literature (Ibelings and Havens, 2008; Beasley and Levengood, 2012).

Cyanobacterial blooms were implicated in bird kills at lakes in Denmark in July 1993 and June-July 1994. These blooms were dominated by *A. lemmermannii* and were shown to contain a neurotoxin with anti-ChE activity (Onodera et al., 1997). Anticholinesterase-producing cultures of *A. lemmermannii* were isolated from the Lake Knud 1993 bloom and gel filtration profiles indicated a similarity between the toxic component from the Lake Knud 1994 bloom with registered bird-kills and anatoxin-a(s) isolated from *A. flos-aquae* NRC-525-17 (Henriksen et al., 1997). Various spectroscopic data indicated that the toxin was anatoxin-a(s). Chemical detection of the same toxin in cultured *A. lemmermannii* also confirmed this species as the cause of death in wild birds (Onodera et al., 1997).

The neurotoxic samples contained equivalents to 20 – 3300 µg of anatoxin-a(s)/g. The highest anti-ChE activities (equivalent to 2300 and 3300 µg of anatoxin-a(s)/g, respectively) were found in samples collected from Lake Knud in connection with bird-kills in 1993 and 1994 (Henriksen et al., 1997). Becker and her co-workers (2010) linked a bloom of *A. crassa* in the Faxinal Reservoir, the main water supply for the city of Caxias do Sul (400,000 inhabitants) in southern Brazil, to the occurrence of anatoxin-a(s) in the water.

Animal poisoning

Toxigenic cyanobacteria are photosynthetic prokaryotes that are most often recognized in marine and freshwater systems, such as lakes, ponds, rivers, and estuaries. Nutrient rich runoff into surface waters, particularly nitrogen- and phosphorus- rich fertilizers, soaps and waste products, has led to significant eutrophication worldwide (>40% in Europe, Asia and America) (Bartram et al., 1999; Smith, 2003). When other environmental conditions are also suitable for their growth, cyanobacteria may proliferate and form toxic blooms in the upper, sunlit layers (Briand et al., 2003). In general, the frequency of blue-green algae poisoning in animals is likely to be underreported due to a lack of methods to confirm exposure (Puschner and Roegner, 2012). In addition, geographical distribution of these case reports are likely biased by available resources. It is probable, however, that blue-green algae poisonings are more common in animals than humans due to a greater direct dependence and contact with surface waters (Puschner and Roegner, 2012). It needs to be pointed out that the reports of anatoxin poisoning in animals are less frequent than microcystin toxicosis, however poisonings have occurred worldwide (Beltran and Neilan, 2000; Edwards et al., 1992; Gugger et al., 2005; Gunn et al., 1992; Yang and Boyer, 2005; Wood et al., 2007; Puschner et al., 2008, 2010).

Since Francis' publication in 1878, numerous case reports describing animal morbidity and mortality exposed to cyanotoxins have been published (Fitzgerald and Poppenga, 1993; Naegeli et al., 1997; Puschner et al., 1998; Gugger et al., 2005; Nasri et al., 2008; Puschner et al., 2008; Wood et al., 2010; Puschner et al., 2010). Specifically, anatoxin-a(s) poisoning has been reported in pigs, dogs, calves, and birds in the US and Europe (Cook et al., 1989c; Mahmood et al., 1988; Onodera et al., 1997). When cyanobacteria implicated in the deaths of nine dogs at Richmond Lake were analyzed, the dominant cyanobacterial species from the water sample was *Anabaena flos-aquae* (Mahmood et al., 1988). The lyophilized bloom material or the high-performance liquid chromatography (HPLC) purified toxin peak, and when administered to mice (i.p), induced clinical signs of salivation, lacrimation, urinary incontinence, defecation, convulsion, fasciculation, and respiratory arrest. Further comparison of the semipurified bloom toxin with an irreversible anti-ChE anatoxin-a(s), produced by *A. flos-aquae strain* NRC-525-17, revealed the bloom toxin and anatoxin-a(s) had similar properties on HPLC and on the inhibition of electric eel AChE (Mahmood et al., 1988).

During the neurotoxic *Anabaena* bloom in Denmark (1993- 1994), many birds were found dead on Lake Knud. Their stomach content contained colonies and single trichomes of *Anabaena*, and anti-ChE activities equivalent to 2.1 – 89.7 µg/kg body weight were detected (Henriksen et al., 1997; Onodera et al., 1997). Based on these results, it appears that the water fowl most likely died from cyanobacterial toxicosis.

Toxicoses associated with ChE-inhibiting algae have been observed in fielding pigs near Griggsville, Illinois, Muscovy ducks near Tolono, Illinois (Cook et al., 1989c) and in dogs in South Dakota, USA (Mahmmod et al., 1988) and Saskatchewan, Canada (Carmichael and Gorham, 1978). Clinical signs observed in pigs included hypersalivation, mucoid nasal discharge, tremors and fasciculations, ataxia, diarrhea, bruxism, dyspnea, recumbency, and terminal nystagmus and cyanosis. Clinical signs in ducks included hypersalivation, regurgitation of algae, diarrhea, tremors, reduced responsiveness and activity, ataxia, polydipsia, dilation of the cutaneous vessels in webbed feet, dyspnea, recumbency, wing and leg paresis, opisthotonus, cyanosis, and clonic or tonic seizures prior to death (Beasley et al., 1989). Clinical signs of cyanobacterial toxicoses in domestic, companion, and wildlife species are described in detail in recent publications by Puschner and Roegner (2012) and Beasley and Levengood (2112). Animals poisoned with anatoxin-a(s) often die within 30 minutes of exposure due to hypercholinergic activity and respiratory failure (Matsunaga et al., 1989; Puschner and Roegner, 2012).

Pretreatment and treatment

The pathophysiologic effects of anatoxin-a(s) were investigated in anesthetized adult male Sprague Dawley rats given the toxin by continuous intravenous infusion until death. Rats pretreated with atropine sulfate (50 mg/kg, i.p) survived significantly longer ($P < 0.05$) than non-atropinized rats, suggesting the involvement of muscarinic receptor associated effects in anatoxin-a(s) - induced toxicity and lethality. Even when survival time of rats was increased by pretreatment with atropine, phrenic nerve amplitude increased, indicating a lack of a depressive effect of anatoxin-a(s) on central mediation of respiration (Cook et al., 1990). In case of exposure to anatoxin-a(s) at a sublethal dose, atropine can be given repeatedly until cessation of salivation. However, it is important to carefully monitor the patient for anticholinergic effects and to reduce or discontinue atropine, should adverse effects develop.

Rats continuously ventilated during anatoxin-a(s) infusion, survived a dose more than 4-fold greater than a consistently lethal dose of the toxin. Thus, the cardiovascular effects of anatoxin-a(s) alone could not be attributed to the death of rats. Electromyographic (EMG) activity recorded from the diaphragms of rats during continuous toxin administration, revealed an increase in muscular electrical activity that became more random and finally decreased prior to death, suggesting a toxin-induced neuromuscular blockade *in vivo*, which was the ultimate cause of death of the anatoxin-a(s) challenged rats (Cook et al., 1990).

Treatments directed toward eliminating poisoning symptoms and *in vivo* protection from anatoxin-a(s) poisonings were investigated using oxime reactivators and atropine or pretreatment with a carbamate and atropine. Anatoxin-a(s) was shown to be an active site-directed inhibitor of AChE, which is resistant to oxime reactivation due to the formation of an adduct on the enzyme (Hyde and Carmichael, 1991). In *in vivo* studies, these authors demonstrated that pretreatment with physostigmine and high concentrations of 2-PAM were the only effective antagonists against a lethal dose of anatoxin-a(s).

Human risk assessment

Cyanobacteria produce toxins that may present a hazard for drinking water safety (Patocka, 2001). These toxins (microcystins, nodularins, saxitoxins, anatoxin-a, anatoxin-a(s), cylindrospermopsin) are structurally diverse and their effects range from liver damage (including liver cancer) to neurotoxicity. The occurrence of cyanobacteria and their toxins in water bodies used for drinking water poses a technical challenge for water utility managers (Hitzfeld et al., 2000). Blooms of toxic cyanobacteria are very common in Brazilian waterbodies as a consequence of eutrophication processes, and anatoxin-a(s) together with other cyanotoxins were detected during a cyanobacterial bloom in the Tapacurá reservoir (Molica et al., 2005).

Cyanobacterial toxins or cyanotoxins are responsible for or implicated in animal poisoning, human gastroenteritis, dermal contact irritations and primary liver cancer in humans. Several incidents of human illness and more recently, the deaths of 60 haemodialysis patients in Caruaru, Brazil, have been linked to the presence of microcystins in water (Rao et al., 2002). Other toxic metabolites of Cyanobacteria, including anatoxin-a(s), represent risks for human health as well (Patocka and Streda, 1989; Mátlová et al., 2004). Mass developments of cyanobacteria in lakes and brackish waters have repeatedly led to serious concerns due to their frequent association with toxins (Dittmann and Wiegand, 2006). The greatest human exposure route to cyanotoxins is believed to be via the water supply, and consequently, the treatment of water containing high cyanobacteria concentrations requires special care. The removal of cyanobacteria cells from an eutrophic water is possible, but it is neither reliable nor cost-effective (De Julio et al., 2010). It is worth mentioning that commercially available blue-green algae dietary supplements are also a potential source of anatoxin-a exposure and thereby can pose risks to human health (Rellán et al., 2009).

Assays

It is clear from the literature, that numerous methods are available for detetion of most cyanotoxins, although many publications on monitoring data indicate that the favored approach is the use of proven, robust methods for individual toxins (Lawton and Edwards, 2008). Determination of anatoxin-a(s) in environmental samples is hampered by the lack of specific methods for its detection (Dörr et al., 2010). Recently, Puschner et al. (2008, 2010) reported an LC-MS based method for quantitation of anatoxin-a in a variety of matrices. A simple and fast method is to detect anatoxin-a(s) using a biosensor based on the electrochemical detection of the activity of AChE. Biosensors using ChEs as the biorecognition component have been used to assay OPs and carbamates for a long time and are very popular (Pohanka et al., 2008, 2009). Among several ChEs, electric eel enzyme was found to be the most sensitive to anatoxin-a(s) and was thus used to build disposable amperometric sensors. The system displayed a detection limit of 1 μg/L anatoxin-a(s). Oxime reactivation was used to discriminate between the toxin and potential insecticides present in the sample (Villatte et al., 2002).

By using mutated enzyme, the sensitivity of detection was brought to below the nmole/liter level. However, anatoxin-a(s) is a natural OP compound, as are several synthetic compounds which are widely used as insecticides. The mode of action of these compounds is via inhibition of AChE, which makes the biotest nonspecific. The use of a four-mutant set of AChE variants, two mutants that are sensitive to anatoxin-a(s) and two mutants that are sensitive to the insecticides, allowed specific detection of the cyanobacterial neurotoxin in environmental analysis (Devic et al., 2002).

Specific and sensitive method for anatoxin-a(s) determination was developed by Rodriguez et al. (2006). The method is based on the direct derivatization of the analyte by adding hexylchloroformate in the alkalinized sample (pH = 9.0). The derivatized anatoxin-a(s) is extracted by a solid-phase microextraction (SPME) procedure, submersing a PDMS fiber in an amber vial for 20 min under magnetic stirring. Gas Chromatograph- Mass Spectrometer (GC-MS) is used to identify and quantify the analyte in the single ion mode (SIM). The calibration curve shows linearity in the range of 2.5-200 ng/mL and the limit of detection (LOD) is 2 ng/mL. This method of SPME and GC-MS analysis can be readily utilized to monitor anatoxin-a(s) for water quality control.

CONCLUSIONS

Anatoxin-a(s) is a neurotoxin produced by the freshwater cyanobacterium *Anabaena flos-aquae strain* NRC 525-17. Anatoxin-a(s) is an irreversible acetylcholinesterase (AChE) inhibitor that binds to the enzyme and renders it unable to hydrolyze the acetylcholine (ACh). Since the ACh is not hydrolyzed, the ion channel is left open, once again destroying muscle function through exhaustion. Anatoxin-a(s) is an organophosphonate (OP), similar in its action to synthetic OP nerve agents, such as sarin or soman, which inhibit ChEs by phosphorylation of the AchE active site. Anatoxin-a(s) is the only known natural OP. If given intraperitoneally to rats, it causes signs of severe cholinergic receptors (both muscarinic and nicotinic)

overstimulation, such as salivation, lacrimation, urinary incontinence, defecation, convulsion, fasciculation and respiratory arrest. The potent toxicity of anatoxin-a(s), LD_{50} approximately 20-50 µg/kg body weight for mice, is attributed to its exceptional anti-ChE activity. Anatoxin-a(s) is strictly a peripheral ChE inhibitor. Atropine, but not oxime reactivators of ChE, may be used in the treatment of anatoxin-a(s) poisoning.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Defence - MO0FVZ0000604.

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