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Aerosolization of cyanobacteria as a risk factor for amyotrophic lateral sclerosis

Elijah W. Stommel *, Nicholas C. Field, Tracie A. Caller

Department of Neurology, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756, United States

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ABSTRACT

Sporadic amyotrophic lateral sclerosis (sALS) is a fatal neurodegenerative disease with no known cause. There are many clues to suggest an environmental trigger for the disease, including reports of conjugal couples and co-localized employees that developed sALS. On the island of Guam,a very high incidence of sALS occurred among the Chamorro natives back in the 1940s and 1950s and has been linked to the neurotoxin beta-N-methylamino-L-alanine (BMAA) that is produced by cyanobacteria that live symbiotically in the roots of the cycad plant, the seeds from which were a staple of the Chamorro diet. It has been shown that BMAA was biomagnified up the food chain from the cycad seeds to the now largely extinct, indigenous flying foxes, a former delicacy of the Chamorro natives. Recent evidence suggests that long term, chronic exposure to low levels of BMAA might cause ALS in genetically predisposed individuals. Many exposure routes to BMAA have been implicated thus far, including consumption of contaminated food and exposure to water harboring cyanobacterial blooms which have the capability of producing BMAA. Aerosolization is a well documented means for bacterial or toxin exposure causing subsequent illness, as in the case of brevetoxins and pulmonary disease and Legionnaire's disease. We hypothesize that some cases of ALS may be related to chronic exposure to the aerosolization of cyanobacteria derived BMAA from cooling towers and might explain the observation of conjugal ALS couples.

Introduction

Despite a tremendous amount of research on the genetics of ALS, little focus has been placed on the etiology of sporadic amyotrophic lateral sclerosis (sALS) which represents the majority of ALS cases. There are many pieces of evidence suggesting an environmental trigger, such as geographic variation of sALS incidence [1]. Other convincing arguments for an environmental trigger for sALS include the description of multiple cases of conjugal ALS amongst genetically unrelated couples in France and in other parts of the world [2–4]. Interesting reports of co-inhabitants of apartment buildings [5] and co-workers working in the same building [6] developing ALS provide potential hints to environmental triggers for ALS. Small case reports such as these do not possess statistical significance, but should not be ignored as they could provide clues to a potential environmental trigger.

History of Guam and initial theory of beta-N-methylamino-L-alanine (BMAA)

In the mid 1940s there was found to be an extremely high incidence rate of ALS and ALS-like conditions (ALS/Parkinsonism dementia complex, ALS/PDC), principally in Guam, where in the early 1950s there was estimated a 100× higher rate of ALS than the average worldwide rate [7–9]. Since that time, the disease appears to have evolved over time to predominantly present clinically as parkinsonism and dementia rather than ALS; current rates of ALS in Guam are now similar to other industrialized countries [10,11]. The chief factor responsible for the declining incidence appears to be ethnographic changes, both social and ecological, brought about by the rapid westernization of Guam, rather than genetics [11]. It was suspected that something in cycad seeds, a dietary staple used by the Chamorro natives to make flour, might be responsible for ALS/PDC [12]. The discovery of a neurotoxic non-protein amino acid, beta-N-methylamino-L-alanine (BMAA) in cycad flour became a prime suspect as a cause of ALS/PDC [13,14]. BMAA in cycad seeds is derived from symbiotic cyanobacteria in coralloid roots of *Cycas micronesica* or possibly also from the cycad plant itself [15,16]. BMAA is mainly concentrated in proteins and was consumed by Chamorro natives through multiple dietary sources, including cycad flour, flying foxes (a type of fruit bat), and other animals that fed on cycad seeds [15,17–19]. The cycad plants and flying foxes are all but gone from Guam now, due to a scale insect (*Aulacaspis yasumatsui*) that killed off the cycad plants and the eradication of flying foxes due to the influx of American firearms. The incidence and prevalence rates of ALS in Guam correlate remarkably to the rise in consumption of flying foxes, and with the subsequent extinction of the animals followed years later by a dramatic decrease in the incidence of ALS [15]. Evidence of a lag time of many years between exposure to a potential causative agent and development of clinical symptoms appears

* Corresponding author. Tel.: +1 603 650 8615; fax: +1 603 650 0458.
E-mail address: elijah.w.stommel@hitchcock.org (E.W. Stommel).

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to exist on Guam as well as a time-limited exposure with an "incubation period" of about 10 years or less.

BMAA in brain tissue

Over the last decade, Cox and colleagues have demonstrated that BMAA in cycad seeds is derived from symbiotic cyanobacteria in the coralloid roots of *C. micronesica* and that BMAA in cycad flour is primarily found in protein-bound form. The consumption of cycad flour, flying foxes, and other animals that fed on cycad seeds by the indigenous Chamorro people led to bio-concentration of protein-bound BMAA up the food chain, leading to the accumulation of BMAA in the brains of Chamorro patients with ALS/PDC (mean concentration of BMAA 627 μg/g) [14,15,17–20]. Another study examining the brains of ALS patients in the United States showed that BMAA had accumulated in the brain tissue of all ALS patients tested (mean concentration of BMAA 268 μg/g), but not in those of patients with Huntington's disease, a genetic neurodegenerative disease (mean concentration of BMAA 11 μg/g) or non-neurological controls (mean concentration of BMAA 41 μg/g) [21]. Huntington's disease is purely a genetic neurodegenerative disease, demonstrating that BMAA does not occur as a byproduct of neurodegeneration. BMAA has also been detected in the brains of Canadian patients with Alzheimer's disease, though at lower levels than those found in the ALS patients (mean concentration of BMAA 107 μg/g) and was not identified in control patients without neurological disease [19]. These findings have been confirmed by a second laboratory (mean concentration of BMAA in Alzheimer's brains 214 μg/g) [21].

Neurotoxicity of BMAA

BMAA binds directly to NMDA and AMPA/kainate receptors, and binding is enhanced when the BMAA is carbamated, which produces a molecule that closely resembles glutamate [22,23]. BMAA induces selective motor neuron (MN) loss in dissociated mixed spinal cord cultures at concentrations of approximately 30 μM [23]. Lobner et al. have shown that the mechanism of neurotoxicity is threefold; binding to the NMDA receptor, glutamate receptor 5 (mGluR5) and induction of oxidative stress [24]. More recently Liu et al. [25] found that BMAA inhibits the cystine/glutamate antiporter (system $X_c(-)$ mediated cystine uptake, which leads to glutathione depletion and increased oxidative stress [25].

BMAA may also be incorporated into proteins and subsequently lead to protein misfolding. The mechanism of BMAA incorporation into protein is not yet known, but a large fraction of BMAA is protein bound (60 to 130 fold greater quantity) compared to what has been recovered from the free amino acid pool [19]. A number of other amino acid analogs have been shown to be incorporated into proteins and can modify cell function [26]. Although it is not known if BMAA causes protein misfolding, there is literature showing that even the regular 20 amino acids can be misincorporated if there is disruption of translational fidelity through the use of low levels of mischarged transfer RNAs (tRNAs), which can subsequently cause protein misfolding in terminally differentiated neurons [27]. Protein bound BMAA could serve as a potential reservoir for future release of the toxin into cells [19].

Despite the known mechanisms of BMAA, there is no viable animal model for ALS [28]. BMAA has previously been shown to be acutely neurotoxic to chicks, rats and monkeys [29]. Spencer et al. [13] found that feeding BMAA to monkeys for up to 13 weeks produced neurological signs similar to ALS, but not consistently enough to be considered a good animal model.

Modes of transmission of BMAA and other cyanotoxins through water

The BMAA hypothesis has generated significant interest in how humans might be exposed to BMAA outside of Guam. The presence of cyanobacterial blooms in freshwater and marine water environments around the world could provide a ubiquitous source of exposure. The ingestion of contaminated food products through cyanobacterial exposure, similar to Guam, is one logical explanation. BMAA has been found in high levels in bottom dwelling marine species such as crab and shrimp in the South Florida Bay, the Baltic Sea and the Chesapeake Bay [30–32], all areas with documented cyanobacterial blooms. There are also a variety of dietary supplements on the market which contain blue-green algae/cyanobacteria, but no one has systematically studied the levels of BMAA or other cyanotoxins in these substances. Drinking water could potentially provide another source of BMAA exposure. Conventional water treatment processes remove cyanobacteria [33], however there are many places in the world where drinking water is removed directly from the source with minimal to no processing or treatment, and these reservoirs can be easily contaminated with cyanobacterial blooms [34]. As an example, roughly 4000 people withdraw water directly from Lake Champlain, a large lake in Vermont, USA with serious cyanobacterial blooms (http://www.lcbp.org/drinkwater.htm).

Another route of exposure that is a logical mode of BMAA exposure is the aerosolization of cyanobacteria. There are many examples of toxin aerosolization leading to human illness. The dinoflagellate, *Karenia brevis*, produces a number of cyclic polyether compounds known as brevetoxins that are aerosolized through wave action of marine waters, causing an asthma like syndrome [35,36]. The marine organism, *Pfiesteria piscicida* can be aerosolized, producing a delirium when inhaled [37,38]. Non-tuberculous mycobacterium intracellulare, a pulmonary and potentially waterborne pathogen isolated from fresh and salt water worldwide, is causally associated with hot tubs and demonstrated to be aerosolized [39,40].

Recreational activities in water bodies that experience toxin-producing cyanobacterial blooms have been shown to generate aerosolized cyanotoxin (microcystin), making inhalation a potential route of exposure [41,42]. The natural aerosolization of cyanobacteria is dependent on many atmospheric conditions, but is well documented both in the field [43] and the laboratory [44]. Laboratory studies have shown that cyanotoxins in water could be transferred to air via a bubble-bursting process [45].

Bubble formation can aerosolize bacteria through wave action, gas formation, temperature changes and precipitation where rain hits the surface of the water. Bubbles can also enhance the amount of bacteria and microalgae put into the air (enhancement factor) [44,46]. Photosynthesis leading to O_2 production and hence bubble formation and wind driven turbulence over water can lead to aerosolization making cyanobacteria particularly interesting in this regard. Studies have shown that MC can be aerosolized by bubbles in the lab [45]. Other studies indicate that recreational activities in water bodies that experience toxin-producing cyanobacterial blooms can generate aerosolized cyanotoxins, making inhalation a potential route of exposure [42]. Absorption of cyanotoxins through the lungs, gastrointestinal tract and nasopharyngeal mucosa could result from this aerosolization. Consequently, it could be hypothesized that white caps on waterbodies containing cyanobacterial blooms could result in aerosolization of BMAA. Saunas and showers delivering aerosolized water contaminated with cyanobacteria could provide other possible modes of exposure.

Existing reports of sALS clusters may have a relationship to aerosolization of BMAA. We have previously reported a cluster of ALS in close proximity to a lake with documented cyanobacteria