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Analysis of the Correlation Between Dietary Exposure of b-N-Methylamino-L-Alanine to Mice and ALS/PDC

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Abstract

This study aims to provide further confirmation on a widespread theory that b-N-methylamino-L-alanine (BMAA) can cause neurodegenerative diseases such as ALS/PDC. BMAA has been thought to cause amyloid deposits and neurofibrillary tangles (hyperphosphorylated tau) in patients' brains, and there two things are two of the commonly accepted criteria in the diagnosis of some neurodegenerative diseases. Three groups of randomly divided laboratory mice will be fed the same diet for a year, with doses of BMAA added to the two experimental groups' diets. Cognitive tests and PET scans will be done throughout the course of the experiment to analyze the effects of the added BMAA. There are 3 types of results that could happen. The results can either prove that BMAA causes amyloid deposits and neurofibrillary tangles and these two things cause a decrease in cognitive abilities, that BMAA causes amyloid deposits and neurofibrillary tangles but these two things are not related to cognitive abilities and neurodegenerative diseases, or that BMAA does not cause amyloid deposits and neurofibrillary tangles. This study, if successful, will provide even more evidence to the theory that BMAA causes amyloid deposits and neurofibrillary tangles and that these two then cause neurodegenerative diseases. It does not, however, provide an explanation of the pathological mechanisms of BMAA, amyloid deposits or neurofibrillary tangles, and future studies on these topics need to be performed in order to confirm the theory.

Keywords

BMAA; Neurofibrillary Tangles; Amyloid Deposits; ALS/PDC; Tau.

1. Introduction

b-N-methylamino-L-alanine (BMAA), an environmental toxin produced by cyanobacteria and accumulated in food chains, is thought to cause neurodegenerative diseases in people whose diets consist of certain products that contain such toxin. Numerous studies have been conducted focusing on the correlation between BMAA and neurodegenerative diseases such as amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC). However, the results of those studies are not in total agreement. Many in vivo experiments done on animals such as vervets, rats and mice have yielded positive results[1], while some studies have found evidence that undermines the theory[2].

The biomagnification of BMAA is quite significant, especially in some Pacific islands such as Guam. The local people of the Chamorro village on the island frequently consume flying foxes (which contain high level of BMAA due to the biomagnification process mentioned) or flour made from cycad seeds (Cycas micronesica) that contain relatively high levels of BMAA, and a large portion of those locals are affected by dementia of varying severity[3].

BMAA selectively kills subpopulations of NADPH-diaphorase positive motor neurons through activating metabotropic glutamate receptors such as mGluR5 and ionotropic glutamate receptors such

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as N-methyl-D-aspartate receptors. BMAA can also be mis-incorporated into proteins as it shares a lot of chemical properties with L-serine, therefore leading to protein misfolding, aggregation, and subsequent apoptosis. This misincorporation has also been thought of as a method of slowly releasing BMAA in the central nervous system, causing chronic exposure even when the patient might have been exposed only to short-term, large quantity of BMAA. It has also been proposed that the misincorporation in animals and other organisms in lower trophic levels is the cause of the bioaccumulation that has been proven to be taking place. Fish that contain high levels of BMAA have a chance of also containing high levels of mercury compounds such as methyl mercury, and past studies have shown that BMAA may strengthen the neurotoxicity of such compounds.

As mentioned above, many in vivo experiments are already conducted, but there are still no definitive conclusions. Therefore, more data collected would still be helpful in determining the exact correlation between BMAA and ALS/PDC. In this experiment, common lab mice (*M. musculus*) will be used to test the effects of BMAA on rodents' brains, as they would be exposed to varying amounts of BMAA throughout the course of the experiment.

I hypothesize that the mice will develop neurofibrillary tangles, will have amyloid deposits, and will display a decrease in their overall cognitive abilities due to the dietary exposure to BMAA, for as mentioned above, many studies done on other mammals have yielded positive results.

2. Materials and Methods

In this study, different experimental groups of mice will be fed a diet with varying levels of BMAA added (210 mg kg^{-1} d^{-1}, [1]150 mg kg^{-1} d^{-1}) with all other conditions kept identical. The control group will be fed the same diet without any addition of BMAA, and corn starch will be used as placebo. The experiment will run for twelve months; tests including radial arm water maze (RAWM), object recognition, and PET scans will be conducted seven times throughout the experiment - before the exposure starts and at the 1 week, 1 month, 3 months, 6 months, 9 months and 12 months mark - in order to track their cognitive abilities as the length of exposure increases. After the last cognitive test, the brain samples of all experimental subjects will be taken and analysed using stains for amyloid deposits and hyperphosphorylated tau (neurofibrillary tangles).

Table 1. All mathematically possible permutations

Amyloid deposits	Neurofibrillary Tangles	Cognitive Abilities	Discussed
0	0	0	+
0	0	1	
0	0	-1	
0	1	0	
0	1	1	
0	1	-1	
0	-1	0	
0	-1	1	
0	-1	-1	
1	0	0	+
1	0	1	
1	0	-1	+
1	1	0	+
1	1	1	
1	1	-1	+
1	-1	0	
1	-1	1	
1	-1	-1	
-1	0	0	
-1	0	1	
-1	0	-1	
-1	1	0	
-1	1	1	
-1	1	-1	
-1	-1	0	
-1	-1	1	
-1	-1	-1	

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3. Possible Results

Table 1 can represent any of the three groups (experimental group with high exposure, experimental group with low exposure, or control group) at any given time with exceptions (0 weeks, 1 week, 1 month, etc., exceptions explained below). Some results will be self-contradictory, and some results will be similar to each other, as this is only the mathematical list of *all* possible permutations.

Representing all the combinations of all three groups and all possible results will require a 4-D model (a group axis, a criteria axis, a result axis and a time axis), therefore only some selected overall results will be discussed in form of language later.

A 1 represents a noticeable amount of amyloid deposits, a noticeable amount of neurofibrillary tangles, or a noticeable amount of increase in cognitive abilities. A 0 represents no significant difference in the given field, and a -1 represents an decrease in cognitive abilities or amounts of amyloid deposits / neurofibrillary tangles. All the baseline data will be based off of the test done before the period of exposure to BMAA. There will not be the possibility of a decrease in amyloid deposits and neurofibrillary tangles until at least one 1 is recorded, since they don't exist in healthy, unaffected beings.

3.1 Possible Results 1 (supports hypothesis)

In both experimental groups, a gradual increase in the amount of amyloid deposits and neurofibrillary tangles can be observed, and a gradual decrease in cognitive abilities can be observed, both after some time since the experiment has begun. The group with a higher daily dose of BMAA has a more severe / faster rate of developing the deposits and tangles and a more severe / a faster rate of decrease in cognitive abilities compared to the group with lower daily dose. The control group sees no or statistically insignificant difference(s) in all three criteria measured.

3.2 Possible Results 2 (supports hypothesis)

There are a gradual increase in the amount of amyloid deposits and neurofibrillary tangles and a gradual decrease in cognitive abilities for both experimental groups some time after the experiment has begun, and there is not a significant difference between the two groups. There are no changes measured in the control group.

3.3 Possible Results 3 (partially supports the hypothesis)

There are a gradual increase of amyloid deposits and neurofibrillary tangles and a gradual decrease in cognitive abilities in the two experiment groups some time after the experiment has begun, but there are also a less significant gradual increase in amyloid deposits and neurofibrillary tangles as well as a less significant decrease in cognitive abilities in similar patterns from the control group.

3.4 Possible results 4 (partially supports hypothesis)

There are a gradual increase in the amount of amyloid deposits and neurofibrillary tangles, as well as a gradual decrease in cognitive abilities, for both experimental groups, immediately after exposure. The control group sees no difference throughout the experiment.

3.5 Possible results 5 (partially supports the hypothesis)

The experimental groups see a gradual increase in amounts of amyloid deposits and neurofibrillary tangles, but no change in cognitive abilities. The control group sees no difference in the physiology of the subjects, but sees a gradual increase in cognitive abilities.

3.6 Possible Results 6 (contradicts the hypothesis)

There are no significant differences observed in all three groups for all three criteria.

3.7 Possible Results 7 (contradicts hypothesis)

There are a gradual increase of amyloid deposits and neurofibrillary tangles and a gradual decrease in cognitive abilities some time after the experiment has begun for all three groups. The experimental groups' data are not statistically significantly different from the control group.

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3.8 Possible Results 8 (contradicts hypothesis)

The experimental groups see a gradual increase in amounts of amyloid deposits and neurofibrillary tangles, but no change in cognitive abilities. The control group sees no difference.

3.9 Possible results 9 (contradicts hypothesis)

The experimental groups see a gradual increase in amounts of amyloid deposits and neurofibrillary tangles, but sees a gradual increase in cognitive abilities as well. The control group sees no difference.

3.10 Possible results 10 (contradicts hypothesis)

The experimental groups see a gradual increase in amounts of either amyloid deposits or neurofibrillary tangles, but no change in the other. There are a gradual decrease in cognitive abilities. There are no changes in the control groups.

3.11 Possible results 11 (contradicts hypothesis)

The experimental groups see a gradual increase in amounts of either amyloid deposits or neurofibrillary tangles, but no change in the other, and no change in cognitive abilities. There are no changes in the control groups.

4. Discussion

Most in vivo studies in the past, whether it be on animals such as mice, rats or vervets, or on human ALS/PDC patients, have shown a correlation between BMAA intake and the occurrence of symptoms. However, there are studies whose findings disprove this theory, such as one that proved that the BMAA content in cycad flour, the main diet of the Chamorro people, after washing is too insignificant to cause anything[2], and one that claimed that preconditioning to small doses of BMAA may actually delay ALS progression[4].

My hypothesis is that BMAA *is* one of the causes of ALS/PDC, and this *will* be proven by the experiments performed on mice, as there is a very logical explanation of the possible pathology of BMAA (described in the introduction).

Possible results 1 and 2 fully support the hypothesis, as they show the positive correlation between BMAA intake and severity of both the physiological abnormalities and the cognitive decrease. The control groups successfully eliminate the possibility that the reason lies in the diet itself and not the added BMAA, and the period of time at the beginning of the experiment where no symptoms nor abnormalities are observed agrees with how ALS/PDC develops over time and not gradually.

Possible results 3 still supports the hypothesis and is still quite consistent with the most likely pathology of BMAA established by numerous past studies. However, the control group failed to support the idea that BMAA is the sole reason for the development of amyloid deposits, neurofibrillary tangles and decrease in cognitive abilities. One possible explanation would be that the diet itself contains a cause for the development of ALS/PDC in the mice. The diet would not be the sole cause and the effects of BMAA would still be proven in this case as the control group's symptoms and physiological abnormalities are less severe than the experimental groups, but this failure in establishing a proper control group would undermine the accuracy and credibility of the experiment.

Possible results 4 seems like a result that fully supports the hypothesis at the first glance, but there is not a proper explanation for why the symptoms started developing almost immediately after the first exposure. The only explanation would be that BMAA is either not the cause or only part of the cause for the development of ALS, and there are other factors that started affecting the experimental groups before the exposure to BMAA started. In order to avoid this outcome from happening, all the mice used in the experiment should be raised in identical environments with conventional diets that are proven by time and generations to not cause any diseases/symptoms.

Possible results 5 seems like it rejects the underlying assumption of amyloid deposits and neurofibrillary tangles affect the cognitive abilities of patients negatively, but the control group's increase in cognitive ability balances it out. There is a possibility that the mice gains experience

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through doing the water maze and object recognition tests, and would naturally get better, as the control group demonstrated. Therefore, the not-increasing of the experimental groups would actually mark a decrease in their true cognitive abilities.

Possible results 6, on the other hand, might completely contradict the hypothesis of BMAA having any effect regarding amyloid deposits, neurofibrillary tangles, or cognitive abilities. However, there could be an explanation. BMAA causes neurofibrillary tangles by mis-incorporating itself into proteins in place of L-serine, causing protein misfolding, aggregation, and eventually tangles that cause neurodegenerative diseases such as Alzheimer's disease, and this has been proven by numerous studies done in the past. However, there are also reports that claim L-serine can inhibit the misincorporation[5]. L-serine is a common amino acid occurring in many foods such as eggs, sardines, and tofu, so there is also a possibility that the misincorporation is being prevented by the abundance of L-serine in the diet of the mice used.

Possible results 7 would completely reject the hypothesis from its basis. The fact that there are symptoms and physiological abnormalities happening and that the control group without exposure to BMAA has them on a level as severe as the experimental groups proves that the reason for the development of those symptoms are not related to BMAA at all, but rather lies in the diet itself, the mice themselves, or some other unknown factors.

Possible results 8 appears very similar to possible results 5, but the difference is that the control group sees no difference in their cognitive abilities, therefore disproving the idea that mice get better at cognitive tests as they take more of it. This result still can prove that BMAA can cause neurofibrillary tangles and amyloid deposits, but cannot support the idea that those two physiological phenomena cause a decrease in cognitive abilities, therefore suggesting that amyloid deposits and neurofibrillary tangles are not the reason behind Alzheimer's, Parkinson's, or other neurodegenerative diseases.

Possible results 9 is an unlikely one, suggesting that amyloid deposits and neurofibrillary tangles actually helps with cognitive abilities. This could only be credited to a flaw in the process of the experiment, as although it has not been fully confirmed that amyloid deposits and neurofibrillary tangles cause neurodegenerative diseases, there are no evidence, or even logical explanations, of these two phenomena helping the 'patient's cognitive ability.

Possible results 10 and 11 both prove that BMAA is responsible for either amyloid deposits or neurofibrillary tangles, but not the other. Their difference is that in possible results 10, the cognitive abilities are also negatively affected, and in possible results 11 the cognitive ability remains unaffected. Since the experimental groups see no difference in their cognitive abilities, we can exclude the possibility that practice makes mice better at running mazes. Therefore, for possible results 10, the physiological criterion that sees a difference would most likely be the cause for the loss of cognition, and for possible results 11, it's either the physiological criterion unaffected by BMAA exposure the cause of cognition loss and ALS/PDC, or that both amyloid deposits and neurofibrillary tangles don't matter to the formation of such diseases.

5. Conclusion

This experiment explores the relationship between amyloid deposits, neurofibrillary tangles, and the symptoms of ALS/PDC as well as whether BMAA through dietary exposure could induce the formations of amyloid deposits and neurofibrillary tangles. Although the idea of amyloid deposits and neurofibrillary tangles being the cause of certain neurodegenerative diseases had been established a long time ago and are even already used in diagnosis of patients, this idea is not yet a fact as the exact mechanism of how it happens have not been totally established, but rather as an experience-determined useful method of diagnosis. The role of BMAA in neurodegenerative diseases is even more uncertain as discussed above, and more studies focusing on the exact mechanism of BMAA, amyloid deposits, and neurofibrillary tangles need to be done.

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