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RESEARCH ARTICLE

The effect of pramiracetam in the myelination of the hippocampus in the BALB/c mouse (*Mus musculus*)

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ABSTRACT

Background: Pramiracetam is being used, particularly by students, to increase cognitive function and enhance learning and memory. There are currently sparse data on the effect of pramiracetam, particularly in the brain. One recent study showed that there is an increased nitric oxide synthase (NOS) in systemically administered pramiracetam. **Aims and Objectives:** To determine if there would be a demyelinating effect in the hippocampus that is a sign of neurological damage due to increasing nitric oxide synthase. **Materials and Methods:** A total of fifteen mice were given 200 and 600 mg/kg dose of pramiracetam for qualitative histological comparison of their hippocampal myelination. After administration with food of the pramiracetam for 75 days, the mice were sacrificed and the brains were then extracted intact, and sagittal sections of the brain were made and stained with the Klüver-Barrera method. Hippocampi were analyzed using a compound microscope to observe for demyelination and neuronal degeneration. **Results:** Based on histological analysis, there were no signs of demyelination in the hippocampus of the *Mus musculus* brain. This may indicate that pramiracetam does not cause demyelination that may be due to increased NO production. **Conclusion:** Pramiracetam may be safe to take at doses of 200–600 mg/kg without any demyelinating effects in mouse.

KEY WORDS: Demyelination; Hippocampus; *Mus musculus*; Pramiracetam

INTRODUCTION

Cognitive enhancers are a class of drugs that may elevate an individual's cognition particularly their thinking abilities, which are considered to be meaningful. In a global study done in 2015, it was determined that at least 1 in 10 healthy individuals has taken at least once stimulants to improved performance whether at work or while studying, with participants from New Zealand, the Netherlands, and Hungary having the most experience in using the drug.[1]

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Nootropics are a class of drugs that act on the central nervous system and increase cognitive performance, as well as act as a neuroprotectant, meaning it protects neurons from injury, disorders, and diseases.[2] Some various drug groups that are approved by the US Food and Drug Administration are racetam, cholinergics, acetylcholinesterase inhibitors, AMPAkines, smart drugs, dopaminergics, nootropic vitamins, and neurohormones.[1] Although the mechanism of action of nootropics is still unknown, it is speculated that they improve cognition be being mildly cholinergic or anti-GABAergic.[3]

Pramiracetam, which belongs to the racetam group, is a nootropic food supplement that can supposedly increase cognitive function and enhance learning and memory, as well as protect neurons in cases of traumatic brain injury.[4] It is a piracetam derivative, which is licensed in Italy as a cognitive enhancer, but its development in the United States has been discontinued.[5] There is evidence that it can increase nitric

oxide (NO) activity in the cerebral cortex[6] and has shown to reverse memory defects in rats with cholinergic deficits, showing possible cholinergic effects.[2] Even though it's mode of action is not clear,[5] several potential mechanisms have been suggested, which includes high-affinity choline uptake[2] and increasing NO synthase (NOS) activity in the cerebral cortex in rats.[6] There has been an interest regarding pramiracetam and other piracetam derivatives in the treatment of disorders related to Alzheimer's disease,[7] cognition, epilepsy, seizures, neurodegenerative diseases, ischemia, and anxiety disorders.[8] Pramiracetam is considered more potent than piracetam, pramiracetam's potency being based on the dosage requirements compared to piracetam and other piracetam derivatives. Pramiracetam has shown to improve cognitive deficits associated with traumatic brain injury[8] and showed antiamnesic effects on volunteers treated with scopolamine, an acetylcholine competitive antagonist, that can cause reversible amnesia.[7] It has been shown that its main action may be attributed to increasing memory and learning by synergistically acting with choline, leading to greater enhancement of cognition.[3] Furthermore, it has been showed that it also regulates the release of glutamate in the cortex and hippocampus, suggesting that it has an effect on N-methyl-D-aspartate (NMDA) receptor function. On the other hand, although it has low affinity to glutamate receptors, it causes AMPA receptor stimulation which leads to influx of calcium in the brain. This leads to the recent reports, suggesting that it is an acetylcholinergic and glutamatergic enhancing drug.[1,3] Despite these findings, there have only been 4 clinical trial publications before 1999 concerning pramiracetam, possibly because their findings produced inconclusive results, thus requiring better-controlled studies, as well as lack of target specificities and exploring possible long-term risks.[8]

The myelin sheath is a phospholipid layer, formed by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system, that facilitates synapses and provides structural and biochemical support for the axons of neurons and speeds up neuronal conduction.[9] The myelin sheath is segmented and interrupted at the intervals by the nodes of Ranvier. The segments are about 0.5–1.0 mm in length. It is the substance that colors the white matter.[10] Myelination is the process of the formation of the myelin sheaths around the axons. It begins in the late fetal development and the forebrains of humans through early adulthood. It has also been suggested that this process may continue up to at least the age of 30.[10] Myelination has been linked to synaptic plasticity, and it has been suggested that it is more than a developmental process and is related to cognitive functions, which is shown in developed white matter structures in children who have increased cognitive function and motor skills.[11] On the other hand, demyelination is the effect of damage or injury done to the myelin sheaths that can lead to slowing down of conduction and transmission of signals in the nervous system.[12] Demyelination can be caused by diseases such as multiple sclerosis[13] or prolonged intake of antipsychotic medications.[14] NO is a free radical that can perform various roles in the human body, like a signaling molecule, and moderator of neuronal functions.[15] NO can be produced in several physiological processes by NOS that converts L-arginine to NO.[9] NO has been implicated to have a role in the myelination of the developing[2] brains as well as being associated with an increase in central nervous system myelination in rats.[16,17] However, NO has been shown to be capable of being toxic to oligodendrocytes and can induce the degeneration of axons that may lead to the process of demyelination.[17,18]

Since neurons are irreplaceable and given the potential of pramiracetam to affect NO levels and the relation of NO to demyelination, it would be a relevant area of inquiry to explore these relationships further. Knowing the effects, particularly whether the increased in NO synthesis will lead to demyelination, will allow a more prudent and scientific usage of the product. Specifically, the objective of the study was to determine whether demyelination would result from the administration of pramiracetam to mice.

MATERIALS AND METHODS

Procurement of Animals

A total of fifteen 8-week-old, weighing around 25–30 g, female BALB/c mice were obtained from the University of the Philippines Manila. The sample size was determined based on the methods of Charan and Kantharia.[19] Animals were then placed in the Animal House at De La Salle University and were housed in individual standard sized cages. Standard commercial rodent food (pellet form) and drinking water were provided. The cages were lined with autoclaved paddy husk and cleaned twice a week. Before the experiment, all mice were acclimatized for 1 week to adapt to an environment with the temperature of 23°C and 55% humidity at a 12 h light:12 h dark cycle. All succeeding experiments in animals were approved by the Institutional Animal Care and Use Committee of De La Salle University (Reference # 2013-005). The experimental procedures were performed from September 2014 to April 2015.

Administration of Treatment

The 15 mice were divided into three groups, a control group and two experimental groups at doses of 200 mg/kg and 600 mg/kg, following the transformed[20] doses from the study of Corasaniti *et al.*[6] Pramiracetam was purchased in powder form and were administered to the mice together with the food for 75 days. At the end of the study, mouse was sacrificed through cervical dislocation, and the brain was harvested for histological analysis.

Histological Preparation and Analysis

The parenchyma of the brain was extracted intact, which included the 3 meningeal layers. The extracted brains were fixed with 10% neutral phosphate-buffered formalin solution. The tissue samples were then brought to St. Luke's Medical Center, Quezon City, for histological processing. Klüver-Barrera stain was used to demonstrate the presence of myelin sheaths of the axons in the white matter of the nervous tissue, as well as demyelination. Histological analysis was performed with a compound light microscope, and photodocumentation was done using a Sony DSC-S930 SteadyShot 10.1 Megapixel digital camera.

RESULTS

Histological analysis showed no demyelination in both the control groups and experimental groups [Figure 1].

The white matter as shown in Figure 1a-c shows no regions of demyelination, which would be seen as white patches in the areas stained blue. Any demyelination on the white matter would be seen as patches of unstained tissue where the white matter (white arrows) would be. Figure 1d-f show the pyramidal cells in the hippocampus. The axons of these pyramidal cells project to the white matter of the hippocampus and are part of the neuronal circuits present in the hippocampus, along with the granule cells of the dentate gyrus as shown in Figure 1g-i, which is densely packed.

We can infer from the results that the white matter of the 200 mg/kg group or the 600 mg/kg group is intact, with the presence of the intact pyramidal cells, and neuronal damage of any significance would affect the myelination of the white matter. This can also be said of the granule cells, as they project to the pyramidal cells, and account for a large amount of the neurons in the central nervous system,[21] indicating that the neural pathways in the hippocampus are intact.

DISCUSSION

The results of the study showed that there is no demyelination in the brain of the mice that may be due to pramiracetam administration. This may have a positive impact on the use of the medication particularly since it is being used for cognitive enhancement. Since no demyelination was seen, it is possible that the dosage of 200 mg/kg and 600 mg/kg was not sufficient to increase levels of NO in the brain. The lack of demyelination suggests that the pramiracetam doses given to the 2 experimental groups had no significant effect on the white matter and consistently show that no demyelination occurred. While the exact mode of action of pramiracetam is still not fully understood, the effects of increasing NO activity may not be sufficient to cause demyelination. Furthermore, since NO at normal levels may promote myelination,[16] it may be inferred that pramiracetam, given the clinical trials that was performed, may actually be protective rather than damaging to neurons.

Cognitive functions have long been thought to require an active participation of the hippocampus. The hippocampus, a three-layered structure in the temporal lobe, has been linked to long-term memory consolidation and considering that

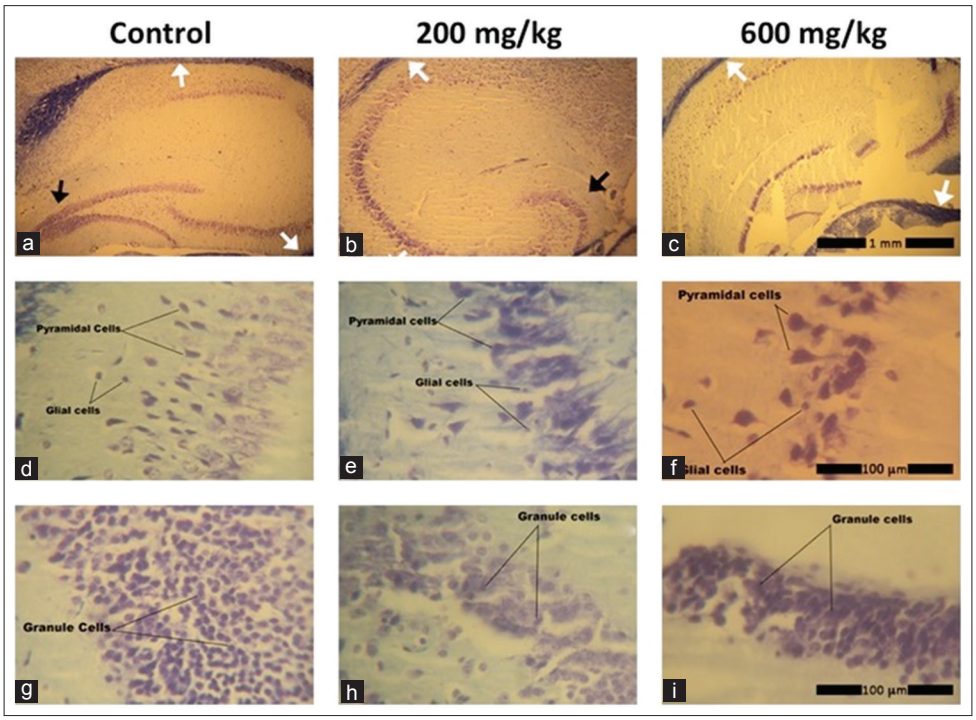

Figure 1: (a-c) Different groups showing the hippocampus with white matter (white arrows) and dentate gyrus (black arrow). (d-f) Different groups showing pyramidal cells and glial cells in the hippocampus. (g-i) Different groups showing showing granule cells in the dentate gyrus

the hippocampus is part of the limbic system along with the amygdala, anterior thalamic nuclei, and the limbic cortex, it can also be associated with learning, memory consolidation, and behavior.[9] It has also been shown to exhibit neurogenesis.[22] Since pramiracetam can affect cognitive functions, it can be inferred that it can have an effect on the hippocampus. The acquisition and retention of memories require an active role of the hippocampus. The cornu ammonis (CA) or horn of Ammon, part of the hippocampus proper, is differentiated to four fields such as CA1, CA2, CA3, and CA4. The pyramidal cells of CA3 and CA4 continue on to the area of the dentate gyrus, which is also part of the hippocampal formation. The hippocampus is also part of a major dopaminergic pathway, called the mesolimbic pathway, which in turn is a part of the mesolimbic/mesocortical system. The mesolimbic or mesocortical system is thought to be involved in behavioral and cognitive responses.[9] Although neurons are amitotic, there has been research that shows neurogenesis in the dentate gyrus of the hippocampus of adult mammalian brains, where they have found neural stem cells.[22]

Pramiracetam, a nootropic racetam derivative drug, has been used for the purpose of increasing cognition, however, with a few clinical evidence to support its claim in long-term memory formation. It has also been assessed in elderly patients with mild cognitive impairment and arterial hypertension. Results of the study showed that improvements in attention, digit span, short-term memory, verbal paired association, and visual reproduction as well as long-term memory were present.[23] In another study on patients with head injury and anoxia, sustained improvement was seen in 18 months of the treatment even up to 1-month post-withdrawal of the drug.[24] The cognitive effects of pramiracetam have somehow been attributed to the accelerated hippocampal acetylcholine turnover, which increases septal-hippocampal cholinergic neuronal impulse flow.[25] One study in rats showed that there is an increasing NO activity in the cerebral cortex of rats given pramiracetam.[6] It has been attributed that NO in the hippocampus may have a role in learning and memory because NMDA receptors are activated during avoidance tasks in chicks.[26] While the presence of NO is normal in the central nervous system, high levels can be toxic to oligodendrocytes and cause axonal degeneration,[17] which is an integral part of demyelination.[13] Overproduction of NO has been seen to induce the generation of highly reactive species such as peroxynitrite and stable nitrosothiols, which may cause irreversible cell damage.[27] Neurotoxicity has also been associated with NO in a variety of neurological disorders including stroke, Parkinson's disease, and even HIV dementia.[28] These effects may be attributed to an increase in different isoforms of NOS all of which may lead to detrimental effects.[29]

It may thus be inferred from this study that there may be different effects of administration of pramiracetam in different test organisms and may thus need further study to verify whether adverse effects may be seen or not. Furthermore, without conclusive evidence on the effect of pramiracetam, careful use of the nootropic is warranted.

CONCLUSION

The results showed no signs of demyelinated nerve fibers and white matter structures and no significant cell damage. The dosages given to the mice were based on a study done on rats given pramiracetam, and the dosage requirements were adapted to mice. The dosages that were given the rats showed an increase in NO activity in the rat cerebral cortex, although in different experimental setups. NO may be a factor in myelination as well as demyelination, but the administration of pramiracetam did not cause any such demyelination in this trial. Pramiracetam at these dosages did not seem to cause demyelination, and despite its potential to increase NO activity, the increased levels of NO, if any, did not cause toxicity.

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