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Relationship between Amyloid-beta 42 Levels and Y-maze Alternation Values in Sprague Dawley Alzheimer's Induction Received Medium-Chain Triglycerides Therapy

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Abstract

BACKGROUND: At present, there is no pharmacological therapy that can cure Alzheimer's disease. Treatment is only limited to preventing progression and controlling risk factors that worsen Alzheimer's. Medium-chain triglycerides (MCT) are nutritional therapies that are being studied to prevent the progression of Alzheimer's disease.

AIM: This study aims to see the effect of giving MCT to the value of percentage alternation Y-maze test and serum A β -42 levels as a marker of Alzheimer's disease.

MATERIALS AND METHODS: This study is an experimental study using postp-test control group design. Samples from this study were 30 Sprague Dawley rats which were divided into positive control groups, negative controls, and three treatment groups. Positive control group and treatment were induced by Alzheimer's by ovariectomy and d-galactose. After induction, MCT were given to the treatment group for 6 weeks. After treatment, the levels of A β -42 serum were examined by ELISA and cognitive function was examined by Y-maze. After that, the data were analyzed by ANOVA. $p < 0.05$ was said to be statistically significant.

RESULTS: The results showed that this study was found a moderate relationship with a positive pattern. This means that the higher the percentage alternation value, the higher the level of A β -42 in serum which indicates that the higher the percentage alternation value, the higher the clearance of A β -42.

CONCLUSION: This study concluded that the group of rats given MCT has a serum A β -42 level higher than the group of rats that were not given MCT.

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Introduction

Medium-chain triglycerides (MCTs) are a fat that has a medium carbon chain length between C₆ and C₁₂. Metabolically, MCT has its own uniqueness, that is, MCT does not enter the lymphatic system and peripheral circulation but is directly transported to the liver through the portal circulation, thus affecting the speed of metabolism to energy [1]. MCT metabolism in some ways is similar to carbohydrate metabolism and produces energy quickly through ketones production. Ketone is the compounds other than glucose that can be used by the brain to produce large and fast energy so it is very beneficial for the aging brain [2].

Ketone bodies can provide alternative energy in Alzheimer's disease. Ketone bodies are usually produced from fat deposits as glucose substitutes in conditions of chronic hypoglycemia, such as during fasting, or when carbohydrate consumption is very low. In humans, ketone infusion can reduce the hormonal response to acute hypoglycemia and can improve

cognitive function [3]. The mechanism of mediating ketone effects on cognitive functions is unclear. Rapid increases in some areas of cognitive function indicate that ketones can function as alternative fuels for brain neuron cells in MCI or Alzheimer's patients. Alzheimer's sufferers show defects in brain glucose metabolism that may arise from many factors, such as A β toxicity or from disorders of lipid homeostasis. Although acetyl-CoA is generally provided for the Krebs cycle of glycolysis, when glucose is not available, β -hydroxybutyrate can function as an alternative fuel for the brain [3].

Other findings in a study with Alzheimer's induction rats revealed that beta-hydroxybutyrate maintains neuronal integrity and stability in rat hippocampus. Case reports in patients with chronic hypoglycemia due to insulin use who have impaired cognitive function, with beta-hydroxybutyrate infusion improved cognitive function in the control group and improved cognitive function related to hyperketonemia in a group of patients who experienced memory disorders. Brain imaging studies also show an

increase in cerebral blood flow by administering beta-hydroxybutyrate in healthy subjects [4].

The pathophysiology of Alzheimer's disease is not fully understood, but the cascade amyloid hypothesis is the most trusted hypothesis as the main cause. Senile plaques formed from amyloid-beta ($A\beta$) deposits in tissue fluid around neurons. $A\beta$ is a fraction of amyloid precursor protein (APP) which normally functions for growth and maintains neuron stability [5]. Amyloid protein precursor (APP) is a transmembrane glycoprotein found in neurons. APP in normal conditions will be described in the cell and cut by three enzymes, namely, α -secretase, β -secretase, and γ -secretase. APP described by the α -secretase will produce a non-toxic amyloid-beta ($A\beta$) fragment. APP which is described by β -secretase will be continued by cutting by γ -secretase so as to produce a soluble and neurotoxic $A\beta$. This toxic $A\beta$ fragment called $A\beta$ -42 is the most toxic form because its ability to form oligomers is faster than other fragments [6-8].

$A\beta$ fragments can stick together and develop into a soluble deposit. Then, the deposit mixes with neuron cells to form plaque fibers that harden, solid, and insoluble, which are toxic to healthy neurons. $A\beta$ is also thought to produce free radicals that interfere with the biochemical balance in neuron cells which disrupts neuronal metabolism [5]. In normal conditions, the amount of $A\beta$ in brain tissue is strictly controlled, starting from the production process, secretion, and degradation of $A\beta$ and cleaning of the brain parenchyma which is regulated by a very controlled process [5]. The $A\beta$ concentration in the tissue is very important for maintaining the structure of this peptide, and increasing its concentration in the tissue is a possible cause of aggregation. Excessive $A\beta$ production and disruption of clearance mechanisms are considered responsible for neuronal damage [9,10].

Materials and Methods

Experimental design

This research is a laboratory experimental study with a post-test only control group design. The samples from this study were 30 Sprague Dawley rats induced by d-galactose and ovariectomy to impair cognitive function. Furthermore, MCT was given. The inclusion criteria were 12-week-old female Sprague Dawley rats weighing 200–250 g who were healthy and who had suffered cognitive impairment. Exclusion criteria were those who experienced physical disabilities and died during the study.

The research subjects were divided into five groups: MCT group dose 1, dose 2, dose 3, positive control, and negative control. Treatment is given for 6 weeks, after that, cognitive function assessment by

Y-maze performed and ELISA of $A\beta$ -42 levels serum using rat $A\beta$ 1-42 ELISA kit (e-EL-R 1402).

Dosage determination

The dose of MCT in human therapy is 10–45 g/day. The dose for mice is obtained by converting a dose of 70 kg to a rat weighing 200 g. The conversion rate of a human with a body weight of 70 kg to a rat weighing 200 g is 0.018. In this study, MCT doses were given, namely, dose I = $0.018 \times 15 \text{ g} = 0.27 \text{ g/day/200 g body weight}$ and dose II = $0.018 \times 30 \text{ g} = 0.54 \text{ g/day/200 g body weight}$ and dosage III $0.018 \times 45 \text{ g} = 0.81 \text{ g/day/200 g body weight}$ [11,12].

Examination of ELISA kit

Add 100 μL standard or sample to each well. Incubate for 90 min at 37°C. Remove of the liquid. Add 100 μL biotinylated detection Ab. Incubate for 1 h at 37°C. Aspirate and wash 3 times. Add 100 μL HRP Conjugate. Incubate for 30 min at 37°C. Aspirate and wash 5 times. Add 90 μL of substrate reagent. Incubate for 15 min at 37°C. Add 50 μL stop solution. Read at 450 immediately. Calculation of results.

The Y-maze method

Testing is done in a Y-shaped maze with three identical arms at a 120° angle from each other. After the introduction to the center of the maze, the animal is given free access to explore the three arms. If the animal chooses different from one it arrived from, this choice is called an alternation. This is considered the correct response while returning to the previous arm is considered an error. The total number of entries and orders is used to calculate the percentage of an alternation. This test is particularly useful in assessing hippocampal damage, quantifying the cognitive deficits in transgenic mice, and evaluating the effects of drugs on cognition [13].

Research ethics

This study was already passed the ethics clearance and has been approved by the Ethics Committee of the Faculty of Medicine, Andalas University, Padang with registration number: 530/KEP/FK/2018.

Results

From Figure 1, it can be seen that the positive control group has the lowest serum $A\beta$ -42 level

compared to other groups. Low levels of Aβ-42 often associated with deteriorating cognitive function.

Table 1: Serum Aβ-42 levels

Group	n	Mean±SD	Minimum	Maximum
Negative control (K-)	6	7168.78 ± 2056.38	4798.67	9897.78
Positive control (K+)	6	2788.21 ± 1832.91	1190.47	5665.97
Dose 1	6	7123.92 ± 1629.40	5172.51	9419.26
Dose 2	6	6002.42 ± 1561.54	3901.47	8492.16
Dose 3	6	6012.39 ± 1169.93	4918.30	7535.14

From Figure 2, it can be seen that the positive control group has the lowest percentage of alternation Y-maze compared to other groups where the positive control group has a percentage alternation value below 50%. It means that the positive control group has impaired cognitive function, while other groups have a normal cognitive function.

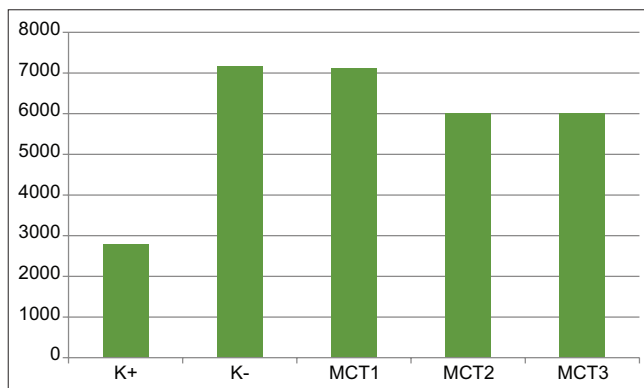


Figure 1: Graph of serum Aβ-42 level

Discussion

Level data Aβ-42 in serum was tested statistically with ANOVA. The results showed that the negative control group showed a significantly different difference in Aβ-42 levels compared to rats in the positive control group. The average level of Aβ-42 in the negative control group was 7168.781 while in the positive control group 2788.209. The positive control group showed a significant difference in Aβ-42 levels for the MCT 1 group, MCT 2, and MCT 3, where the mean levels of each were 7123.925, 6002.421, and 6012.389. Between the MCT group receiving different doses, there are no differences, and the mean results of Aβ-42 are significant (Table 1).

Table 2: Average Y-maze value between groups

Group	Mean±SD	p
Negative control (K ⁻)	76.42 ± 5.40	0.000
Positive control (K ⁺)	41.25 ± 7.09	
Dose 1	71.91 ± 5.20	
Dose 2	69.26 ± 10.57	
Dose 3	70.11 ± 4.97	

The Y-maze test results also showed a negative control group, MCT 1, MCT 2, and MCT 3, having alternation percentage values above 50% and a

positive control group having an alternation percentage value of <50%. Comparative test results show that the higher the Y-maze value, the higher the serum Aβ-42 level (Table 2).

Table 3: Comparison of the mean percentage alternation between groups

Groups	p
K ⁻	
K ⁺	0.000
Dose 1	0.795
Dose 2	0.408
Dose 3	0.531
K ⁺	
K ⁻	0.000
Dose 1	0.000
Dose 2	0.000
Dose 3	0.000
Dose 1	
K ⁻	0.795
K ⁺	0.000
Dose 2	0.963
Dose 3	0.991
Dose 2	
K ⁻	0.408
K ⁺	0.000
Dose 1	0.963
Dose 3	1.000
Dose 3	
K ⁻	0.531
K ⁺	0.000
Dose 1	0.991
Dose 2	1.000

Animal experiments before have shown that MCT can penetrate the blood-brain barrier and be oxidized in astrocytes under ketogenic conditions. Hence, MCT can provide a direct or indirect source of energy for the brain through the production of ketones [1]. Other data indicate the potential for an MCT diet to treat neurological disorders, especially Alzheimer’s disease. Animal experiments showed that the administration of MCT reduced Aβ levels in frontal dementia dog brain biopsy and increased mitochondrial respiration [14].

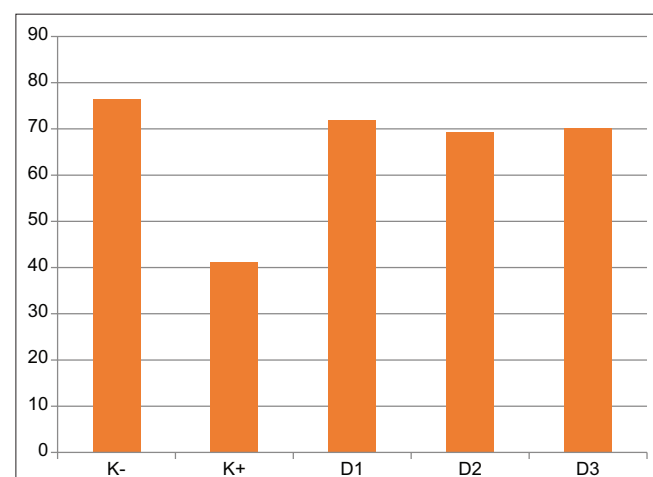


Figure 2: Graph of result Y-maze

Studies of transgenic Alzheimer’s rats given a ketogenic diet with MCT supplementation showed that after 1 week, there was a significant increase in beta-hydroxybutyrate levels. The cognitive function and cortical Aβ-42 levels were measured after 6 weeks of MCT; the results showed that rats that received MCT had significantly lower levels of Aβ-42

than rats that did not receive MCT supplementation but did not show differences in cognitive function in the two groups [15].

Recently research has concluded, MCT increases brain metabolism by supplying ketones without affecting brain glucose utilization. Ketones from MCT compensate for brain glucose deficits in people with Alzheimer's directly after ketones reach plasma [16]. In this study, the effect of giving MCT to the levels of A β -42 serum and Y-maze of Sprague Dawley rats with neurodegenerative disorders has been shown to be significant. This can be seen from the comparison of the levels of A β -42 in all groups. The results of the statistical test showed that there were significant differences in the levels of A β -42 between the positive control group and negative controls. However, there were no significant differences between the negative control groups and the three treatment groups. However, among the three treatment groups, it was seen that serum A β -42 levels were not significantly different.

Conclusion

From the results, it can be concluded that the group of rats given MCT has serum A β -42 level higher than the group of rats that were not given MCT. Serum A β -42 levels describe beta-amyloid clearance in the brain, where if there is an imbalance in production and clearance, fractions of beta-amyloid will form oligomers that are toxic to brain neurons and buildup of beta-amyloid oligomers can develop into senile plaques, which further worsens cognitive function disorders. The greater the level of A β -42 in the serum means the better the A β clearance. This is reflected in the percentage value of alternation. The better the clearance, the better the cognitive function of rats.

In this research, the value of Y-maze, there was a significant difference between the positive control group and the negative control group and the treatment group. While between the negative control group and the treatment, there was no difference in the percentage of alternation and in the A β -42 serum value, there was also a significant difference between the positive control group and the negative control group and the three treatment groups.

After doing this research, it can be suggested that MCT can be an alternative energy source for glucose replacement in Alzheimer's patients so that it can slow the progression of the disease.

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