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The Alteration of Cognitive Function in Iron Overload Mice

Abstract—Regular blood transfusions is a lifetime treatment for blood disorder such as thalassemia and it can lead to the iron accumulation within the organs. Iron accumulation in the brain can cause toxicity by increasing reactive oxygen species (ROS) and altering apoptotic signal. However, the impact of the iron overload in cognitive function is still unclear. The purpose of this study is to investigate the effects of iron overload to the cognitive function of mice. Three groups of mice were divided into three groups with different dosing of iron injections (0, 0.1, and 0.3 mg/mice). Iron was injected intraperitoneally for 19 days. A special experimental maze was used to assess the cognitive function. The test was repeated three times; before injection, the 6th day of injection, and the 11th day of injection. After 19 days of injections, brain weight was measured and brain histology examined. Our results showed that cognitive function was impaired after iron injections. Cognitive function test indicated that the time required by group 1 during the first test were 265.20 ± 47.11 seconds, during the second test were 123.20 ± 18.33 seconds, and during the third test were 151 ± 45.80 seconds. Next, the time required by group 2 during the first test were 254.60 ± 44.16 seconds, during the second test were 176,60 ± 32.54 seconds, and during the third test were 259.60 ± 63.28 seconds. Then, the time required by group 3 during the first test were 260.20 ± 44.90 seconds, during the second test were 241.20 ± 32.65 seconds, and 272.40 ± 65.79 seconds during the third test. The data analysis indicated insignificant changes between group 1, group 2, and group 3 with p-value 0.068. There were no significant changes in brain weight and brain histology among all groups. We conclude that iron overload can cause alteration of mice cognitive function without change in brain histology

***Keywords*—Cognitive function, iron overload, special experimental maze**

1 INTRODUCTION

According to Nagelhout [1], Thalassemia is a genetic disease caused by inherited mutations. It decreases either α-globin or β-globin synthesis chains in composing adult hemoglobin leading to anaemia, tissue hypoxia, and red cell hemolysis. It also relates to the imbalance in globin chain synthesis. Sir et al. [2] reported that the prevalence of thalassemia carriers in Southeast Asia is about 25-30%. Routine blood transfusion is a long-time treatment for these conditions. However, Hussain et al. [3] demonstrated that long-term blood transfusions would increase the serum iron level. According to Merono et al. [4], iron overload is defined as increase in iron deposition with or without tissue destructions. According to Whitney and Rolves [5], free iron is able to act as ~~a~~ free radical, could attack cell membrane, protein, and DNA.

White et al. [6] demonstrated that the iron excess could induce toxicity of brain cells by increasing Reactive Oxygen Species (ROS) which is one of the oxidative stress sources and could initiate the apoptotic signal. Intracellular iron accumulation could be harmful towards some proteins, such as Ca^{2+}ATPase, a glutamate transporter, $Na^{+}K^{+}$ATPase, ceramide, NMDA receptor, and sphingomyelin. Synapse dysfunction and necrosis of neuron could be caused by abnormalities in these proteins.

According to Blasco et al. [7], iron accumulation in some parts of the brain, such as the caudate nucleus, hypothalamus, and lenticular nucleus could decrease the cognitive function of the brain. Tucker et al. [8] demonstrated that iron excess could cause changes in the left hemisphere electroencephalography which is related to the cognitive function of the brain.

The mechanism underlying alteration of cognitive function due to iron excess remains uncertainty. The aim of this study is to investigate the effect of iron excess on the cognitive function of mice.

2 METHODS

2.1 Animals

This study was approved by Health Research Ethics Committee Faculty of Medicine Universitas Padjadjaran, Bandung, West Java, Indonesia. All experiments were conducted based on 3R and 5F principles.

The subjects of the study were 15 male mice (*Mus musculus)* aged 10-12 weeks which were purchased from Biofarma Company, Bandung. They were placed in a room with 12/12 h light, dark cycle, adequate air circulation and water supply. They were also adapted to the surroundings for seven days trained three times for cognitive function test before the experiment.

Sample size was calculated using resource equation method. According to Charan and Biswas [9], value E is calculated based on decided sample size. The value if E should lies within 10 to 20 for optimum sample size.

E = Total number of animals - Total number of groups.

In this study we used 15 mice which divided into 3 groups. Therefore, the value E was 12 which means the sample size was optimum.

2.2 Grouping of Experimental Groups

The subjects were divided into 3 groups based on the dose of iron injection:

a. Group 1 (control) : intraperitoneal 0.2ml saline injection;
b. Group 2 : 0.1mg intraperitoneal iron dextran injection;
c. Group 3 : given 0.3mg intraperitoneal iron dextran injection.

Each group was injected with saline or iron dextran intraperitonially with different dose for 19 days.

2.3 Cognitive Function Test

Before injection, the mice were adapted three times for special experimental maze of cognitive function test called modified hole board. According to Labots et al. [10], modified hole board is used to assess multiple dimensions of unconditioned behaviour, mainly in mice and rats. The test was conducted in modified hole board with a dimension of 50 cm x 50 cm x 50 cm. In the middle of the maze, a 35 cm x 22 cm x 1 cm board with 10 cylinders was placed. The diameter of each cylinder is 3 cm with 1 cm in height (Figure 1). The maze was made from grey polyvinyl chloride and equipped with bottle cap.

Memory component of mice was evaluated by this test. A piece of almond was placed in the middle of the three cylinders covered by coloured paper. After that, the mice were placed in the middle of the cage and taken to complete the test was measured from the time the mice entered the cage until the time when the mice recognized the third almond. The cognitive function test was conducted in the same way as the adaptation technique and performed in three time lines which was day 0, 6, and 11. The tests were performed on all 15 mice

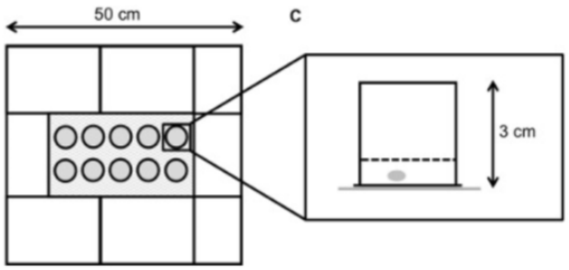

Figure 1. Special experimental maze for cognitive function test. The test was conducted in modified hole board with a dimension of 50 cm x 50 cm x 50 cm. In the middle of the maze, a 35 cm x 22 cm x 1 cm board with 10 cylinders was placed. The diameter of each cylinder is 3 cm with 1 cm in height.

2.4 Organ Harvested

After 19 days, the mice were sacrificed by cervical dislocation technique and their brains were taken. The brain's weight was measured by a scale and then stored in the container tube with formaldehyde solution after coronal section.

2.5 Hematoxylin & Eosin (HE) Staining of Brain

The brains were deparaffinized and then stained with Hematoxylin solution. After rinsed with running water, it was counterstained by Eosin solution. Histology of brain tissue were examined under the light microscope.

2.6 Statistical Analysis

The results were analysed with IBM SPSS Statistics 20 using repeated analysis of variance (ANOVA) to evaluate the differences between groups with p-value <0.05 was considered as statistically significant.

3 RESULTS

3.1 Brain Weight

Brain weight measurement showed that the brain's weight of group 1 was 0.39 ± 0.06 gram,

while group 2 was 0.39 ± 0.04, and group 3 was 0.41 ± 0.03 gram.

The mean brain weights of the mice were shown in Figure 2 and there was no significant difference in mean brain weight between group 1, 2, and 3 (p = 0.707).

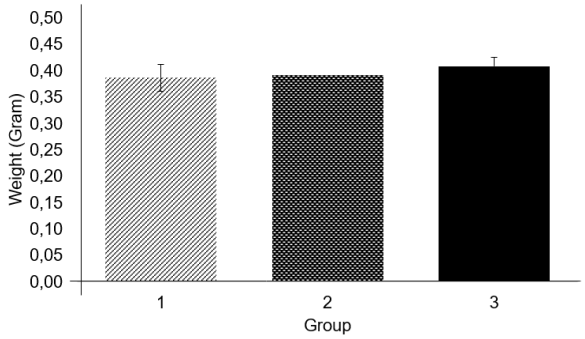

Figure 2. Brain weight curve of of (1) 0.2ml saline injection (2) 0.1mg iron injection (3) 0,3mg iron injection. There were no significant changes in brain weight among all groups.

3.2 Cognitive Function

Cognitive function test indicated that the time required by group 1 during the first test were 265.20 ± 47.11 seconds, during the second test were 123.20 ± 18.33 seconds, and during the third test were 151 ± 45.80 seconds. Next, the time required by group 2 during the first test were 254.60 ± 44.16 seconds, during the second test were 176,60 ± 32.54 seconds, and during the third test were 259.60 ± 63.28 seconds. Then, the time required by group 3 during the first test were 260.20 ± 44.90 seconds, during the second test were 241.20 ± 32.65 seconds, and 272.40 ± 65.79 seconds during the third test. Thus, the tests indicated that the time required for ~~a~~ group 1 decreased during the second test and then increased during the third test, meanwhile the time required for group 2 and group 3 increased during the second and third test.

The data analysis indicated insignificant changes between group 1, group 2, and group 3 with p-value 0.068. However, the curve indicated some changes in the cognitive function of the whole group. The recorded time in iron injected groups was almost two times higher compared to the control group (Figure 3).

3.3 Brain Histology

There were no significant changes in brain histology among all groups (Figure 4).

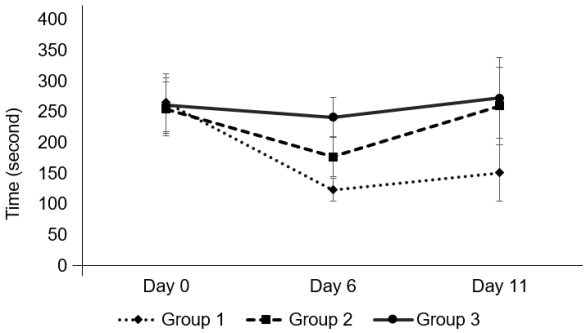

Figure 3. Cognitive function curve of (1) 0.2ml saline injection (2) 0.1mg iron injection (3) 0,3mg iron injection. The recorded time in iron injected groups was almost two times higher compared to the control group.

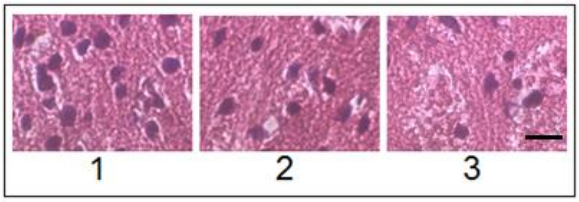

Figure 4. HE staining of brain in (1) 0.2ml saline injection (2) 0.1mg iron injection (3) 0,3mg iron injection. There was no significant changes in brain histology. Scale bar=100 µm

4 DISCUSSION

The aim of this study is to investigate the effect of iron excess on the cognitive function of mice. Cognitive function tests indicated that the time required for a group 1 is decreased during the second test and then increased during the third test, meanwhile the time required for group 2 and group 3 increased during the second and third test. The cognitive function alteration could be due to the increase in reactive oxygen species (ROS) and intracellular iron accumulation that causes synapse dysfunction and necrosis of neuron [6].

According to Hare et al [11], iron enters the brain through Fe_2Tf uptake into brain capillary endothelial cells (BCECs) by endocytotic mechanism. Iron becomes segregated from Tf inside the endosome. After that, transferrin (Tf) binds to Tf receptors and come out of the brain through the blood brain barrier. Segregated Fe^{2+} come out of the endosome through DMT1 before accumulated in the labile iron pool.

White et al. [6] demonstrated that the iron excess could induce toxicity of brain cells by increasing Reactive Oxygen Species (ROS) which is one of the oxidative stress sources and then initiate the apoptotic signal. This could be related to alteration of cognitive function that most obviously seen in group 3.

The limitation of this study is that we were unable to determine whether olfactory sensation factor in influencing the findings. This study illustrated that iron overload may lead to deterioration of cognitive function. We recommend future studies to investigate the effects of iron overload onto the cognitive functions of thalassemia major patients who required regular blood transfusion. We also recommend future studies to analyze iron overload effects on other functions of the brain as iron overload may leads to brain toxicity.

5 CONCLUSION

Iron overload can cause alteration of mice's cognitive function without changes in brain weight and brain histology.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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