

ROLE OF MELATONIN IN EXPRESSION OF MALONDIALDEHYDE ON MICROGLIA CELLS OF RAT INDUCED HEAD INJURY

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Background: brain injury is condition that harm human life. This study examines the application of melatonin in reducing oxidant status and barriers to the formation of cerebral edema in a rat brain injury model. The main purpose of this study is to prove the role of melatonin on the expression of Malondialdehyde (MDA) and histological injury in a rat head injury model. **Methods:** This study was a randomized experimental posttest only control group design. This experimental was carried out on male Sprague Dawley strain *Rattus norvegicus*, aged of 10-12 weeks, and weight of 300 g. Rat brain injury model was performed based on Marmarou (1994).¹ Histology were observed using hematoxylin-eosin staining and immunohistochemistry, MDA was assessed using antibodies specific to each MDA protein. Observation and calculation of immunohistochemistry studies were also performed. **Results:** In this study, histological observation area covers an area of bleeding, number of immune-competent cells and the diameter of the arteries. Histology observation results showed that there is a significant reduction in diameter of arterial blood vessels of the brain injury tissue. Immunohisto-chemistry results showed that there is a significant reduction of MDA expression amount microglia cells of brain injury tissue. **Conclusion:** From this study, it can be concluded that Melatonin is a potent hydrogen peroxide scavenger that reduce the production of MDA.

Keyword: human; life; staining; scavenger

INTRODUCTION

Head injury is one of dangerous condition for human live. Each year in United State of America there are 1.500.000 cases of head injury. Around 50.00 of them are dead and 80.000 of them have defect after discharged from hospital. Currently in United State of America there are 5.300.000 people with disability caused by head injury.² There are no accurate report about head injury cases in Indonesia. Therefore, efforts should be carried out to provide data of patient with manifold, hyperventilation and hypertonic liquid.

After primary head injury, many chemical reactions take place for survival the injured cells. These chemical reactions sometimes have a bad impact known as secondary head injury. The secondary head injury is indicated by cerebral edema. Unwell treated of this condition causes increase of intracranial pressure and leads to ischemia on brain cell and even end up to death. The head injury caused damage of cell. This will be followed phagocytosis of debris cell by macrophage (monocyte on tissue) and resulted in inflammation.³ During inflammation respond, lipopolysaccharide induced monocyte to secrete

interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor (TNF)- α .⁴ TNF- α induced reactive oxygen species (ROS) on endothelial so the endothelial will excrete e-selectine. E-selectine is adhesion molecule and neutrophil⁴ and IL-8 is neutrophil protractant or neutrophil chemotactic factor (NCF) that induced neutrophil migration to periphery⁵ so the cell can be bound by e-selectin in the surface of endothelial. Interleukin 1 has an important role in induce the endothelial to secrete vascular cell adhesion molecule (VCAM) as adhesion molecule to monocyte.⁶

Melatonin has a role as antioxidant,⁷⁻¹¹ biological modulator for mood, sleep, libido, reproduction system, Circadian rhyme¹² and immunoregulator.¹³ In addition, melatonin has a function as anticonvulsant. Any study reported that melatonin is neuroprotective in head injury,^{7,14-15} ischemic head injury,^{16,17} alzheimer.¹⁷ Melatonin also minimize the vascular leakage on brain and retina when there is damage of retina blood barrier.^{18,19}

The characteristic of Melatonin antioxidant is its capability in cleaning the free radical and induce the expression of antioxidant enzyme.²⁰ Activity and expression of antioxidant enzyme such as superoxide dismutase (SOD), catalase, glutathion peroxidase and glutathion reductase increase by Melatonin.²¹⁻²⁵ The recent study indicates that Melatonin has a role in reducing lipid peroxidation.²⁶ Melatonin inhibits hypoxia through

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prevention of accumulation of free radical excess.²⁷ Melatonin also minimizes oxidative damage during ischemia and reperfusion.^{15,28}

Melatonin supplementation is effective to minimize the oxidative stress on head injury because of reducing MDA content of plasma as a lipid peroxidation marker. In addition, Melatonin also improves mitochondria function in ischemia and reperfusion. Mitochondria dysfunction resulted in over production of ROS.³⁰ The improvement of mitochondria function by Melatonin will minimize the mitochondria oxidative stress.³¹ Nevertheless, mechanism of Melatonin on disturbance of cerebral edema has not yet clear. Therefore, this study aims to find out role of Melatonin on the minimization of MDA expression of microglia cell after head injury on experiment rat.

MATERIALS AND METHOD

This is a true experimental study applying randomized post only control group design. Samples in this study were *Rattus Novergicus* strain *Sprague Dawley*, male, age of 10-12 weeks and weight 200-300 g. Estimation of sample size was estimated based on Federer, the number of sample is 9 experiment animals for each group. Minimal number of sample for each group is 10 rat. Total required number of experiment rat is 30.

The male of *Sprague-Dawley* that fulfill the requirement is classified into 3 treatment group randomly, i.e. control group, treatment group (group without and without Melatonin), treatment group with ketamine anesthesia, and to make the head injury by craniotomy with coroner incision to anesthetized rat. After durameter is exposed, the weight for 20 gram was fall from the elevation of 20 cm on the areas. The treatment group was treated with Melatonin injection with dosage of 2.5 mg/kg body weight, intraperitoneal one hour after the head injury processed. And the dosage was repeated each 12 hours so total dosage of 7.5 mg/kg body weight. The treatment group without Melatonin after head injury process is stitched without Melatonin application.

RESULTS

This research studies the role of Melatonin in myeloperoxidase enzyme barrier. Experiment was carried out using the head of *Rattus norvegicus* injury model as experiment animal. The role of Melatonin and its relation to cerebral edema process were described.

All data observed were normally distributed and their variances were also homogenous with $p > 0.05$.

Histology condition for observing the effect of Melatonin application to the rat model with head injury was presented in the following figure (Figure 1-4).

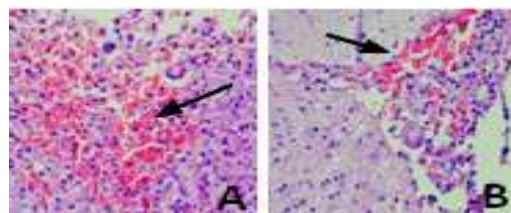


Figure 1
Histological Condition of Cerebral Formation Edema. Post head injury (A) and application of Melatonin (B).

Figure 1 shows that the expose of Melatonin post head injury will minimize the appearance of cerebral edema. The depiction of cerebral edema area is signed by hemorrhagic (arrow), in post head injury. The area of cerebral edema is smaller after expose to Melatonin.

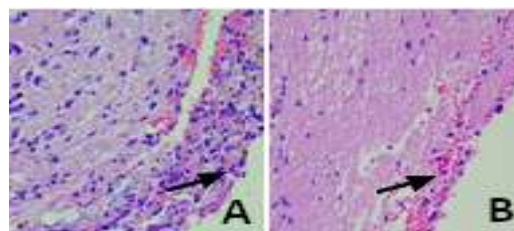


Figure 2
Histological of distribution of immunocompetent. Cell post head injury (A) and application of Melatonin (B)

Figure 2 shows that there is the lower of distribution of immunomicrocompetent cell (arrow). The distribution of immunocompetent cell of the microglia cell in post head injury indicates the lower of number after expose to Melatonin (Figure 2B).

Microglia is assumed as immune cell of central nervous system and treatment of head injury will influence the number of microglia cell. The distribution of microglia cerebral tissue is observed using immune hystochemical method with specific antibody to integrin.

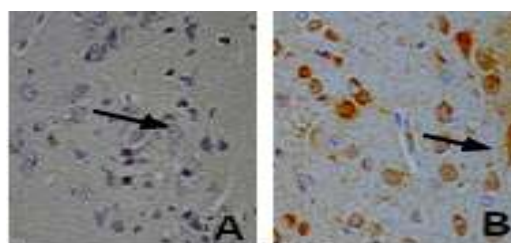


Figure 3
Depiction of Immunohistochemical of Microglia Cell of Cerebral Tissue in Post Head Injury (A) and Application of Melatonin (B).

Figure 3 shows that there is lower of distribution of microglia cell of cerebral tissue in post head injury with Melatonin expose (arrow).

The assessment of MDA content in cerebral tissue was conducted by TBAR method with colorimetric system. The average and standard deviation of MDA content of tissue for each group is shown in Table 1.

Table 1
Average of T-test of MDA content in cerebral tissue

Groups	MDA ($\mu\text{mol/L}$)	<i>p</i>
Control	0.529 (0.129)	0.0001
Treatment-1	2.503 (0.523)	
Treatment-2	0.954 (0.559)	

Remarks: Treatment-1 = treatment with melatonin, Treatment-2 = treatment without melatonin. T-test shows a significant different of MDA content ($\mu\text{mol/L}$) of brain tissue in post head injury.

Table 1 indicates treatment group with Melatonin provide a good effect in lower content of MDA of cerebral tissue in post head injury.

The assessment of MDA in this research is also conducted by immunohistochemical method on location of heat injury. This assessment aims to see the expression of MDA of microglia cell of cerebral tissue of experiment animal with head injury in Melatonin expose.

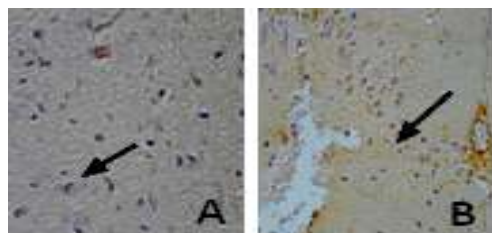


Figure 4
Depiction of Immunohistochemical MDA Expression of Microglia Cell of Cerebral Tissue in Post Head Injury (A) and Application of Melatonin (B).

Figure 4 shows that there is lower of VEGF expression of cerebral tissue in post head injury with Melatonin expose (arrow). MDA is distributed on microglia I with immuno-staining method it indicated that Melatonin expose to the mouse with head injury will minimize the number of microglia cell that express MDA (arrow).

DISCUSSION

Most head injury caused by accident. The victim was under 40 years old which was still in a productive ages. Each year in developed countries, there are 10 million people in hospital caused by

head injury. Head injuries are the main factors as a cause of mechanical brain cell injury and trigger the secondary damage after the primary damage. Secondary non mechanical injury is progressive and occurred since the first hour on the first day of post head injury. This research aims to study the using of Melatonin in reducing the oxidant status and obstacle in cerebral edema forming on head injury rat model.

Experiment animals in this research is *Rattus norvegicus* strain Sprague Dawley with the average of body weight of 290.07 (10.48) g. Therefore, the different of expression of dependent variables is caused by influence of induction of head injury or the expose of melatonin. The concept of head injury in this study is based on lesion or injury of brain tissue that did not as a results of degenerative process or congenial. It can be said, the head injury is due to external factor that cause the lower of change of awareness status.

Head injury model in this study was carried out according to Marmarou method.³⁸ Histologically, it observes the hemorrhagic condition, distribution of immune-competent cell. Melatonin is applied to the experiment animal for 3 times with a dosage of 2.5 mg/kg body weight on 1 hour since the induction of head injury and repeated after 12 hour. Therefore, total dosage is 7.5 mg/kg of body weight.

The head injury triggers any mechanism that distributed of secondary injury, i.e. cerebral edema.³⁶ Cerebral edema is the increasing of intracellular cerebral fluid accumulation and or extracellular.⁵¹ This condition is indicated by inflammation of brain tissue with the progressive increasing of cerebral fluid content caused by ischemia, trauma,⁵² tumor,⁵³ and inflammation.²¹ By using injury area analysis indicates the different decreasing (Figure 4.1) on the induction group of head injury with melatonin compared to induction group of head injury with melatonin.

Melatonin (N-asetii-5-methoxytryptamine) is molecule with antioxidant characteristic, cytoprotective and has role in immunomodulator. In the early, the molecule is produced exclusively by pineal gland. But the synthesis of Melatonin had found in the different location of the organism and the main source of Melatonin extra pineal is body immune system. The ischemia process follows the head injury caused the accumulation of lactate acid caused by anaerobe glycolysis, the increasing of membrane permeability, and forming of edema. This process was followed by depolarization of cell membrane and over release of neurotransmitter glutamate and aspartate, activation of N-methyl-D-aspartat, α -amino-3-hidroxy-5-methyl-4 isozazo-propionate, and the change of voltage dependent on Ca^{2+} and Na^{+} channel. The increasing of Ca^{2+} and Na^{+} influxes orderly trigger the intracellular

catabolism process. Ca^{2+} activates lipid peroxidation, protease, and phospholipase that increase the concentration of free fat acid intracellular and free radical (ROS).

This research also proved that the reducing of MDA did not only occurred on microglia cell, but also in cerebral tissue, especially in the area of induction of head injury by measure the content of MDA brain homogenate that indicates the lower of MDA content (μM) significantly on the brain tissue after expose to melatonin.

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