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## **SHORT TERM INDUCTION OF LPS DAMAGES THE HIPPOCAMPUS GYRUS THROUGH APOPTOSIS ON GLIAL CELLS MICE**

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### **Abstract**

*Sepsis is a condition that causes the highest mortality in the ICU. Sepsis occurs due to an infectious process that causes damage to organs such as the brain. During the sepsis process, the brain plays a full role in the body's defense, so that it manifests as encephalopathy. However, the mechanism in short-term sepsis not fully describes. This study aims to examine brain damage to the hippocampal gyrus in experimental animals induced by LPS in a short time. Methods: Mus musculus mice aged 8 weeks were divided into three groups namely control group (Ctrl), induction of LPS for 4 h (LPS-4h),*

*and induction of LPS for 8 h (LPS-8h). LPS was injected by intraperitoneal with 25 mg/kg. The brain damage was seen histologically with HE staining. Results: The results of LPS injection showed an insignificant increase in leucocytes among all groups. Whereas the histological analysis found that glial cell damages increased significantly ( $P < 0.05$ ) with time dependency after LPS induction compared with the control group. Conclusion: Short-term induction of LPS destroys the hippocampal gyrus via high glial cells.*

## **Keywords**

LPS, Sepsis, Hippocampus, Microglia, Inflammation

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## **1. Introduction**

Neuroinflammation plays an important role as an innate immune response in the central nervous system because it plays a role in balancing normal and abnormal conditions. All diseases associated with damage to the CNS are broadly inflammatory processes of nerves characterized by activation of microglia and astrocytes in nerves (Negi and Das, 2018). Also, this activity has been proven in animal and human experiments where microglia, astrocytes, the activity of lymphocyte infiltration, and activation of other inflammatory mediators were found. As is well known, microglia play an important role in the immune control of nerves due to the damage process and play a role in the production of neurotropic and neurotransmitters, so microglia are very important components of the nervous system (Frost & Schafer, 2016).

The neuroinflammatory process is not only caused by the mechanism that comes from the nerve itself but can be caused by the septic process of infection as a result. As in previous studies showing that sepsis causes cerebral dysfunction due to systemic inflammatory response in infection, causing encephalopathy through activation of the microglial (Sonneville et al., 2013). Inflammatory signals to which microglia respond include the cytokines IL1 $\beta$ , IL6, and TNF $\alpha$  (Parkhurst et al., 2013). The previous study has revealed much about the pathophysiology of sepsis. However, sepsis is a very complex disease process because it involves many systems in the body including coagulation, immunity, metabolism, and endocrine and ROS balance as the main pathway for the inflammatory process (Steckert et al., 2015). The increase in proinflammatory causes an increase in cerebral arteriolar pressure (40-200uM) which reduced the cerebral blood flow (Gotts & Matthay, 2016). Previous studies on animals with stroke models have shown that cerebral hypoperfusion causes cerebral microvascular damage, neuropathological processes, and cognitive dysfunction which are the main causes of neurodegeneration (Hofman et al., 2013). However, sepsis-induced brain damages

in incompletely understood. Therefore, this study was conducted to determine how the image of brain damage in the hippocampus of mice induced by LPS, as a model of sepsis.

## **2. Methods**

The procedures used in the present study are described below.

### **2.1 Animals**

This study used *Mus musculus* mice at the age of 8 weeks, that we purchased from Pusvetma laboratory. Before being given the treatment, the experimental animals were acclimatized for 7 days in a cage at room temperature and given a normal diet and drinking. All research methods have been approved by the Ethics Committee of Hang University (I/032/UHT.KEPK.03/VI/2020). All procedures for conducting research in accordance with the implementation instructions in the laboratory.

### **2.2 Animal Group and Study Design**

Twenty-four mice with ten weeks of age were randomly divided into three groups. The first group was the control group (Ctrl), the second group was the LPS injection group for 4 hours (LPS-4h), and the third group with the LPS injection for 8 hours. LPS induction was given as much as 25 mg/kg per mouse by intraperitoneal injection.

### **2.3 LPS Administration**

The lipopolysaccharide from *Escherichia coli* purified by phenol extraction is obtained from sigma Aldrich company (Sigma-Aldric Co., St. Lous, MO, USA) with serial number O111: B4.

### **2.4 Histological Analysis**

After retrieval of brain organs, fixation was carried out using 10% formaldehyde, and then embedded in paraffin. After that, 5  $\mu$ m thin sections were prepared and then stained by hematoxylin and eosin in a block form. Histological analysis was performed under a microscope at 40x magnification.

### **2.5 Statistical Analysis**

Data were analyzed using IBM SPSS statistics with version 25. The variables were reported as mean  $\pm$  standard deviation and median  $\pm$  minimum-maximum. To see a comparison of more than 3 data using one-way ANOVA. The student's *t*-test was used when two means of the group were compared. P values of  $<0.05$  were considered statistically significant.

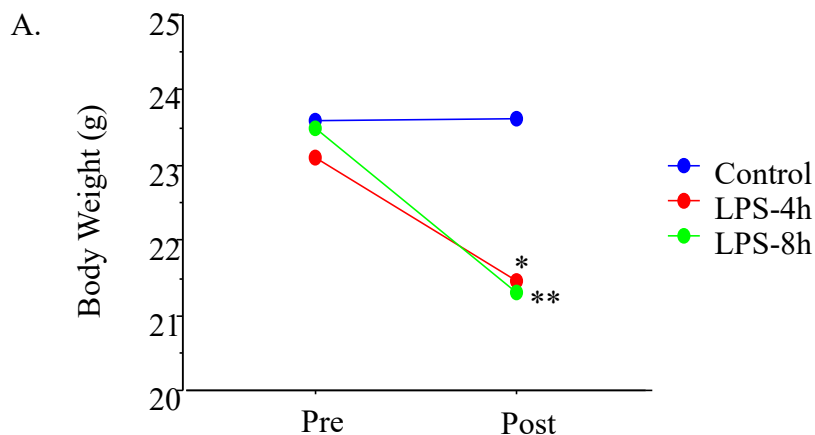
### 3. Results and Discussion

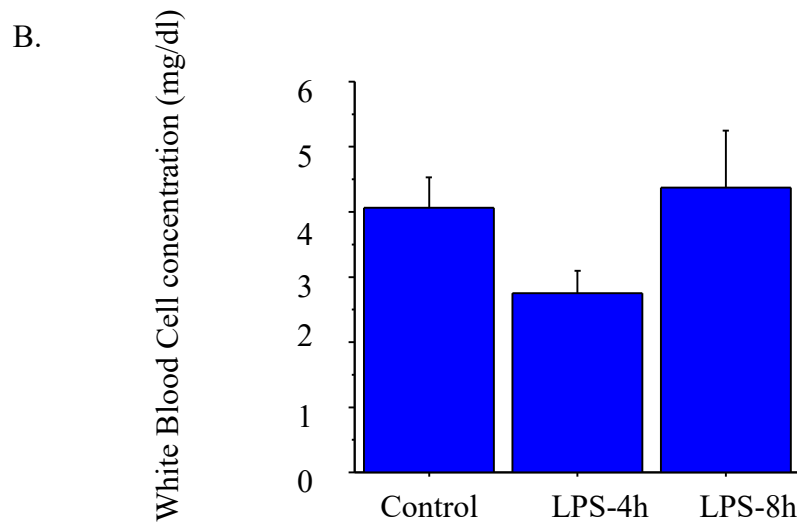
The results from the present study are given below

#### 3.1 LPS-induced Body Weight Loss and Concentration of Leukocytes

The initial development of the sepsis process occurs characterized by the release of endotoxin from bacteria. Where these endotoxins can activate cytokines and inflammatory mediators, causing organ damage, including nerve cells in the brain. LPS induction has been known to cause changes in parameters in the body, especially blood laboratory. In this study we evaluated the LPS injection was effect to made sepsis, white blood cells measured. Figure. 1A showed that LPS injection increased the leukocytes concentration in the blood significantly ( $P < 0,05$ ) in the 4 h and 8 h group compared with the control group. On the other hand, LP-induced sepsis decreased bodyweight significantly ( $P < 0,05$ ) (Fig.1B). The results in this study are in line with the previous study, where it was found that LPS induction had significantly reduced body weight (Lee et al., 2018).

As we know, sepsis is defined as a syndrome characterized by physiological, pathological, and biochemical abnormalities caused by infection (Singer et al., 2016). Based on the guidelines for the diagnosis of sepsis, laboratory tests also determine the criteria for sepsis, one of which is white blood cells (Levy et al., 2010). Interestingly, this study showed that white blood cells count is lower after LPS injection at 4 h duration compared with the control group. Meanwhile, the LPS injection group at a duration of 8 hours showed almost the same results as the control group (Figure.1B). The results of this study are in line with previous studies, they found that the levels of leukocytes after injection of LPS at a duration of 6 hours were lower than the duration of 12 hours (Yates et al., 2011). However, the hematologic response in humans and mice was described with different alterations in lymphocytes and neutrophils (Yokoo et al., 2012). Although leukocytes are known to produce pyrogenic cytokines, the inflammatory response due to LPS induction in this study is not only due to changes in the blood leukocyte profile but can be caused by other sources.





**Figure 1:** Changes in the Concentration of Leukocytes and Bodyweight

(A) Body weight was decreased in the LPS group, (B) White blood cell concentration after LPS induction decreased in 4-h and increased in 8-h ( $n=8$ ).  $^*p<0.05$ ,  $^{**}P<0.01$  versus control values are mean  $\pm$  SEM.

### 3.2 Brain Changes after LPS-induced Sepsis in Histology

Based on the theory of the mechanism of sepsis, several theories have been proposed, including the theory of microorganisms that directly infect the central nervous system, the result of a microorganism product that enters the CNS, the presence of metabolic disorders that affect CNS function, the presence of blood-brain barrier processes, disorders neurotransmitter and receptor distribution and impaired perfusion of brain tissue (Angus & Van der Poll, 2013). All of these theories are concerned with the mechanisms of damage to the central nervous system.

Damage to the hippocampus of the brain is seen from the amount of damage to the glial cells. To examine the morphology of damage glial cells, histopathologic analysis of the section stained with hematoxylin and eosin revealed that the morphology of glial cells in the gyrus hippocampus. As we can see in figure.2A that normal glial cells more abundant in the control group compared to the treatment group with LPS injection. A representative in the figure. 2A is shown in the figure. 2B, which shows that glial cell damage increases with the length of the LPS injection. We can see that the LPS injection at 4 h and 8 h duration increased significantly compared to the control group.



mean of data from eight animals in each group is presented, with SEM by vertical lines. \*\* $p < 0.01$  versus control.

These results indicate that a severe acute inflammatory process has occurred due to the injection of LPS. Acute response to invasive pathogens in the host usually causes macrophages to produce pro-inflammatory and cytokines that can trigger cytokine storms and activate the innate immune system (Raymond et al., 2017). Furthermore, as has been explained in previous studies which explain that there are histopathological changes in organ dysfunction in sepsis, where changes in the central nervous system are damaged in white matter bleeding and hypercoagulability, micro abscess formation, central pontine inflamed myelin, multifocal necrotic leukoencephalopathy, metabolic changes, ischemic changes, and apoptosis (Garofalo et al., 2019).

Previous studies have shown that microglial activation plays a central role in the neuroinflammatory process involving inflammatory mediators such as cytokines, reactive oxidative species (ROS), and neurotransmitters (González et al., 2014). In addition, previous studies have shown that cytokine administration both peripherally and centrally can cause behavioral changes. and other studies have shown that increased TNF-mediator and stereotype scores in sepsis model mice correlate with behavior change (González et al., 2014).

#### **4. Conclusion**

In conclusion, the study of apoptosis glial cells in the hippocampus is important to better understand the pathogenesis of brain dysfunction in sepsis. LPS-induce sepsis in this study has explained that microorganism as the pathogen has a strong correlation to develop cerebral dysfunction by increasing inflammations. That has been shown by increased apoptosis of glial cells in the gyrus hippocampus. The limitations of this study are that it does not examine a dominant inflammatory mediator as one of the factors causing glial cell apoptosis in the brain. So, that further research is needed to ascertain the inflammatory mediators that play an important role in causing brain dysfunction.

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