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ANIMAL RESEARCH INTENSIVE CARE

The effect of ketamine on Kupffer cell count in Wistar rat (*Rattus norvegicus*) model of sepsis

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Abstract

Background: Sepsis is a serious health problem and is associated with life-threatening complications, such as liver dysfunction, renal failure as well as cardiorespiratory disease. Kupffer cells are immune cells that contribute to the pathogenesis of liver dysfunction. This study aims to determine the effect of ketamine on the number of Kupffer cells in experimental animal models of sepsis.

Methodology: This experimental study used fecal-induced peritonitis (FIP) to create a sepsis model in rats (*Rattus norvegicus*). Thirty rats were divided into six equal groups, namely the negative control group, which was not treated with FIP (Group K−), the positive control group or the sepsis model with FIP treatment (Group K+), and the treatment group; the sepsis model rats (FIP treatment) given 5 mg of ketamine/kg intraperitoneally once at 0 h (Group A), at 3 h (Group B), 5 h (Group C) after induction of sepsis with FIP, and every 2 h after induction of sepsis with FIP for four hours (Group D). Kupffer cells were counted six hours after FIP induction using a hematology analyzer. Statistical test was carried out using the One-Way ANOVA test using SPSS 18.0 software.

Results: The number of Kupffer cells in sepsis groups (K+) was significantly higher (22.60 cell/ml) than in the negative control group (K-) (12.68 cell/ml) (P= 0.01). The administration of ketamine in the sepsis model group significantly decreased the number of Kupffer cells close to normal. The number of Kupffer cells was 12.56 cells/ml in Group A, 12.92 cells/ml in Group B, 9.75 cells/ml in Group C, and 8.50 cells/ml in Group D.

Conclusion: The administration of ketamine decreased the number of Kupffer cells in the rat model of sepsis induced by FIP to close to the normal group.

Abbreviations: APP - acute-phase protein; FIP - fecal induced peritonitis; ICU - Intensive Care Unit; IL – interleukin; KCs - Kupffer cells; ROS - reactive oxygen species; NO - nitric oxide; SIRS - systemic inflammatory response syndrome; TNF-α - tumor necrosis factor-alpha.

Keywords: Immunomodulator; Ketamine; Kupffer cell; Sepsis

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1. Introduction

Sepsis is the most common cause of death in the Intensive Care Unit (ICU).[1] In the United States, the number of hospitalizations for sepsis patients is higher than for heart infarction or stroke.[2] The incidence rate of sepsis reaches 535 cases per 100,000 population and is continuously increasing over time.[3] The mortality rate for sepsis in hospitals is 25–30%.[4] The annual cases of sepsis in the ICU RSUD Dr. Saiful Anwar, Indonesia, reached 168 patients, and 78 (46.4%) of them died.[5]

Liver dysfunction and failure are complications of severe sepsis, and contribute to disease progression and mortality.[6] During the early phase of septic shock, liver perfusion becomes impaired, resulting in direct hepatocellular injury.[7] Kramer et al. stated that liver dysfunction is an early sign of sepsis and is an independent risk factor for poor outcomes, based upon clinical results and experimental data.[8]

Kupffer cells (KCs), which constitute the largest population of mononuclear phagocytic cells in the body, account for 80-90% of the total number of natural macrophages and 20% of nonparenchymal liver cells.[9] Kupffer cells have high phagocytic activity and produce cytokines and chemokines.[8] Kupffer cells are considered to be the primary defense against bacteremia and endotoxemia. However, in the case of sepsis, Kupffer cells and granulocytes trigger bactericidal activity, which causes liver damage.[10] Kupffer cells release cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-12, and IL-18, reactive oxygen species (ROS), and nitric oxide (NO) that induce cell injury to endothelium, and hepatocytes.[11] Lipoproteins and teichoic acids derived from Gram-positive bacteria can also activate Kupffer cells and induce liver injury. After lipopolysaccharide stimulation, there is a rapid increase in hepatic messenger RNA IL-6 produced by Kupffer cells, hepatocytes. The combination of IL-6, IL-1β, and TNF-α markedly increased acute-phase protein (APP) gene expression through transcriptional activation.[12]

Loix et al. stated that, as an anesthetic, ketamine not only prevents exacerbation of inflammation but also inhibits inflammation when it is already running.[13] Persson et al. state that ketamine inhibits TNF-α gene expression IL-6 and NO synthetase enzyme induction activity to protect against inflammation.[14,15] The ability of ketamine to reduce NO synthetase and the nuclear factor kappaB (NF-kappaB) as an anti-inflammatory is expected to reduce systemic inflammatory response syndrome (SIRS) due to sepsis. So that intraperitoneal administration of ketamine is expected to reduce the number of Kupffer cells in the liver, contributing to reduced liver cell damage and the resultant morbidity and mortality due to sepsis. This study aimed to determine the effect of ketamine on the number of Kupffer cells in experimental animal models of sepsis.

2. Methodology

This study used a true experimental laboratory design with the randomized post-test-only controlled group design method. The research was conducted at the Parasitology Laboratory and the Anatomical Pathology Laboratory, Faculty of Medicine, Brawijaya University, to examine variables.

The study used *Rattus norvegicus* species of white rats (Wistar strain) as experimental animals. Inclusion criteria included male sex, age 5 months, body weight 200-250 grams, had not undergone any treatment or had no chemical intake, and were in good health (marked by active movement and no noticeable hair fall). Exclusion criteria included sick or injured rats and failure of fecal induced peritonitis (FIP) induction.

The rats were acclimatized for one week by feeding a normal diet consisting of comfeed PARS, wheat flour, and water. The diet was given as much as 40 g per rat daily and given during the day from 12 - 2 pm. After acclimatization, 30 rats were divided into 6 groups, namely the negative control group, which was not treated with FIP (Group K−), the positive control group or the sepsis model with FIP treatment (Group K+), the treatment group, namely the sepsis model rats (FIP treatment) injected ketamine 5 mg/kg intraperitoneally at 0 h (Group A), 3 h (Group B), 5 h (Group C) after induction of sepsis with FIP, and after induction of sepsis with FIP followed by every two hours for four hours (at 0, 2, and 4 hours) (Group D), respectively. This dose was determined based on research by Sun et al.[16]

FIP is a method of making a model of sepsis by injecting fecal fluid at a dose of 1 mg/g of body weight of rats. Fecal fluid was prepared by mixing feces from the rat colon with normal saline until 200 mg/ml.[17] Assessment of the degree of sepsis in rats was done by using the Murine Sepsis Score (MSS).[17]

Dissection of mice was performed 6 h after the FIP injection. Mice were anesthetized using chloroform. The rats were placed on their backs, and the entire surface of the abdomen was doused with 70% alcohol. The abdomen was then dissected, and blood was taken with a 5 ml syringe through the heart. The number of Kupffer cells was counted using a hematology analyzer.

Statistical analysis

The normality test of the data in this study was carried out with the Shapiro-Wilk test. The statistical analysis was performed using the One-Way ANOVA test by SPSS 18.0 software (IBM, USA).

3. Results

The assessment of MSS showed that K+, A, B, C, and D groups had a MSS of more than 1 (Table 1). This finding indicated that FIP successfully induced sepsis

Tabel 1: Murine Sepsis Score

Groups	Murine Sepsis Score (mean ± SD)
Negative control (K−)	0 ± 0
Positive control (K+)	11 ± 1.0
Group A	12.2 ± 1.09
Group B	11 ± 1.0
Group C	10.8 ± 0.84
Group D	12.4 ± 1.52

in rats. The results showed that FIP significantly increased the number of Kupffer cells from 12.68 cell/ml (K− group) to 22.60 cell/ml (Group K+) (P = 0.01).

The administration of ketamine in the sepsis model significantly reduced the number of Kupffer cells close to normal. The numbers of Kupffer cells in the ketamine-treated group were 12.56 cells/ml (Group A), 12.92 cells/ml (Group B), 9.75 cells/ml (Group C), and 8.50 cells/ml (Group D), respectively. Based on statistical results, the number of Kupffer cells in the ketamine-treated groups (Group A, B, C, and D) did not significantly differ from the negative control group (K−) (Figure 1).

4. Discussion

Kupffer cells under physiological conditions act as the first innate immune cells and play an important role to protect the liver from bacterial infections.[18] On the other hand, Kupffer cells under pathological conditions, such as sepsis, contribute to the pathogenesis of sepsis through the production of proinflammatory mediators.[19]

A significant increase in the number of Kupffer cells in the sepsis groups from this study supports the results of the study by Kolios et al.,[11] who suggested that Kupffer cells were increased in cell culture induced with lipopolysaccharide.[20] However, this increase will be followed by a decrease in phagocytic activity, and the production of superoxide will further increase the potential for more infection.[21] Kupffer cells act as effector cells of the immune system and destroy hepatocytes in bacterial infections. Hutchins et al. explained that in sepsis, Kupffer cells have the potential to increase sinusoidal liver cell damage by mediating programmed cell death-Ligand 1.[22] The administration of ketamine in the sepsis groups significantly reduced the number of Kupffer cells close to normal and it had no significant difference with the negative control (normal) group (P > 0.05). According to Yan et al. depleting Kupffer cells is able to protect the liver from inflammation-induced injury and reduce the inflammation in sepsis.[23] The downregulation of immunity could be beneficial since the pathogenesis of sepsis has often involved excessive immune inflammation.[24] Kupffer cells are needed by the body to fight bacterial infections through phagocytic activity. However, the high phagocytic activity of Kupffer cells potentially causes damage to the host tissues. Kupffer cell activation in sepsis is also associated with systemic inflammatory responses and leads to multiple organ failures.[25]

The results showed the lowest number of Kupffer cells in the rats model of sepsis treated with ketamine every 2 h until 4 h. Based on these results, ketamine showed immunosuppressive activity against Kupffer cells. Research conducted by Takahashi et al. showed that ketamine could inhibit the phagocytic activity of Kupffer cells.[26] Ketamine can also increase the survival rate of LPS-induced experimental animals. This study confirms that the administration of ketamine can also reduce the number of Kupffer cells.

Ketamine, an anesthetic agent, has immune-inhibitor properties,[27] and possibly can regulate the number of Kupffer cells in patients with sepsis. Only a limited number of studies have analyzed the effect of ketamine on the number of Kupffer cells in sepsis. Most studies only focused on the effect of ketamine in modulating the immune system.[28] This study shows great insight into Kupffer cell modulation in sepsis patients by using an anesthetic agent. Inhibition of Kupffer cell activation potentially becomes sepsis target therapy. However, clinical studies need to be done on human subjects to confirm the results of this animal study.

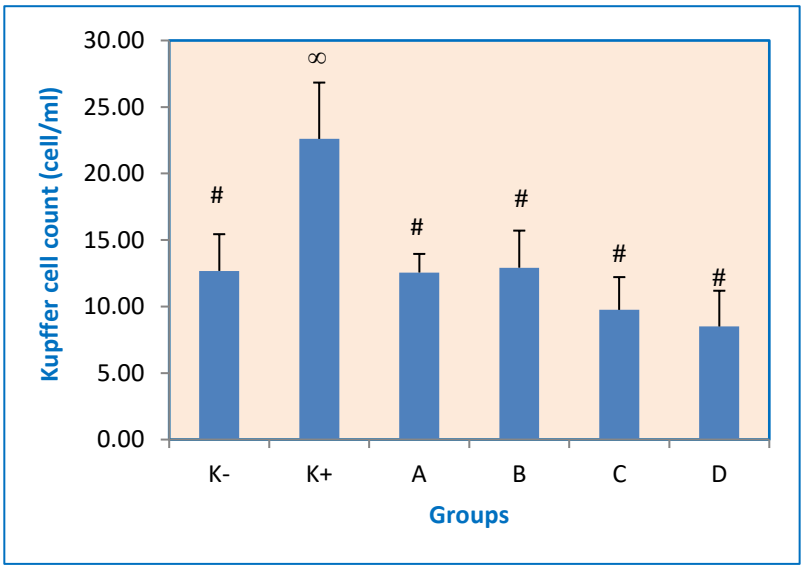

Figure 1: The administration of ketamine decreased the number of Kupffer cells in the rat model of sepsis
[∞ indicates statistically significant difference; # indicates no significant difference]

5. Limitation

There was no dose variation of ketamine used in different groups of this study. Therefore, further research might be done by using more varied doses of ketamine and comparing the effects.

6. Conclusion

Administration of ketamine at different time intervals can cause a decrease in the number of Kupffer cells in rat models of sepsis close to normal. Ketamine possibly can be used in septic patients to prevent organ damage.

7. Ethical approval

The study protocol was approved by the Ethical Clearance Committee of the Faculty of Medicine, Brawijaya University (No. 452/EC/KEPK/12/2016).

8. Data availability

The numerical data generated during this research is available with the authors.

9. Conflict of Interest

The authors report that there were no competing interests to declare.

10. Author's contribution

AAA: Supervision, concept, design of the study, conduction of study, manuscript preparation

WJ: Conduction of study, data analysis, manuscript preparation and editing

ANW: Conduction of study, manuscript preparation, manuscript revision, data curation

RML: Conduction of study, manuscript revision, final approval

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