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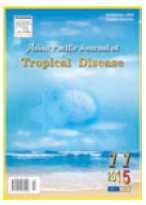

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The effect of ketamine on the lipopolysaccharide-induced inflammation in *in vitro* culture of HUVEC

Aswoco Andyk Asmoro*, Ramacandra Rakhmatullah, Scarpia Puspitasari, Khairunnisai Tarimah, Siti Chasnak Saleh, Mochammad Aris Widodo, Edi Widjajanto

Medical Faculty, University of Brawijaya, Malang, East Java, Indonesia

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ABSTRACT

Objective: To report the effect of ketamine treatment on inflammatory cytokines induced by lipopolysaccharide.
Methods: Human umbilical vein endothelial cells culture was used in this research. The measurement of inflammatory cytokines was performed by using ELISA.
Results: The results showed various levels of inflammatory cytokines. The nuclear factor-κB results were unstable, but interleukin 6 and tumor necrosis factor-α showed similar results. The expressions of interleukin 6 and tumor necrosis factor-α decreased in all observation times.
Conclusions: In general, our results of human umbilical vein endothelial cells culture were supported by other researches related with the effect of ketamine in suppressing inflammatory effect induced by lipopolysaccharide. Ketamine could suppress inflammatory cytokines in all observation times.

1. Introduction

Sepsis is a clinical syndrome which occurs as a manifestation of immunological inflammation process caused by excessive body response to stimulation from microorganisms' products[1]. There are almost 13 million people around the world suffering from sepsis every year and approximately 4 million people died of sepsis. Severe sepsis and septic shock have the highest mortality rate up to 46%[2]. In sepsis, the inflammation process triggers apoptosis[3]. In sepsis and multi-organ dysfunction syndrome, the process is initiated by the release of cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β induced by lipopolysaccharide (LPS), and in turn increases the intracellular calcium and free radicals which trigger apoptosis[4]. TNF-α and IL-1β were released in the first 30–90 min after LPS induction and continued with the activation of inflammation cascade[5-7].

Treatment with ketamine was reported to increase the survival rate of rats, the animal models for sepsis. The mechanism was suggested as inhibitory effect of ketamine on pro-inflammatory cytokine IL-6[8]. Ketamine was proven to suppress the activation of nuclear factor-κB (NF-κB) and TNF-α in the 1, 4, and 6 h of observation time after LPS induction in rats with sepsis condition[9]. Besides, other researches also reported that ketamine could suppress the toll-like receptor 4 and NF-κB activities in the 1, 3, and 5 h after LPS induction[10].

Herein, we reported the effect of ketamine treatment on inflammatory cytokines induced by LPS. In this research, we used the *in vitro* culture of human umbilical vein endothelial cells (HUVEC) with monocytes.

2. Materials and methods

2.1. Sample preparation

Samples were taken from healthy patients who were already given the informed consent. Immediately after birth by cesarean section, the umbilical cord was cut approximately 20 cm and put directly into the cord solution without washing. Isolation and culture of HUVEC were carried out for less than 4 h after child birth.

*Corresponding author: Aswoco Andyk Asmoro, Medical Faculty, Brawijaya University, Malang, East Java, Indonesia.
Tel: +628123252730
E-mail: aandykasmoro@gmail.com

2.2. Isolation and culture of HUVEC

Isolation of HUVEC culture was performed by using collagenase treatment which was similar to the previous methods with some modifications[11,12]. Isolated endothelial cells were cultured in 24 cm^2 tissue culture wells with cover glass inside and coated with 0.2% gelatine. Cultures were incubated in 5% of CO_2 incubator under the temperature of 37 °C until cobblestone-like appearances were formed.

2.3. LPS and ketamine treatment

Confluent cultures were induced with 1 μg/mL of LPS. Ketamine was given immediately after LPS induction. The concentration of ketamine used in this research was 50 μmol/L. Observations were conducted in three different incubation times (0, 1 and 3 h). Before LPS and ketamine-induction, HUVEC was co-cultured with monocytes to enhance the triggering mechanism of LPS.

2.4. Cytokines levels analysis

Cytokines levels were analyzed by ELISA. ELISA method was performed based on manufactured protocol. The measurements included several cytokines, such as NF-κB, TNF-α and IL-6.

2.5. Statistical analysis

Statistical analysis was conducted by One-way ANOVA test continued with Duncan test. All measurements were performed with SPSS 19.0 for windows with significance $P < 0.05$.

3. Results

ELISA analyses of NF-κB showed various results of three different treatments (Figure 1). The highest level of NF-κB (20.10%) was obtained after 1 h of LPS induction. The inhibitory effect of ketamine was shown by lowering NF-κB level to 7.16% 1 h after induction. One interesting result was observed in 3 h after induction in which culture induced with ketamine and LPS had significantly higher NF-κB level than that of culture induced with LPS only. Ketamine induction resulted in unstable levels of NF-κB.

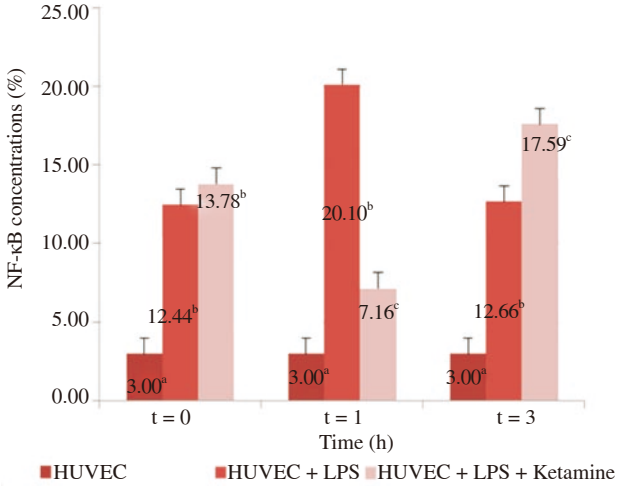

Figure 1. ELISA result of NF-κB measurement.
Different letters mean there is a significant difference among groups.

Different result was shown from measurement of IL-6 level (Figure 2). Constant decreasing level of IL-6 was observed at all time of observation in culture induced with LPS and ketamine. The levels of IL-6 after induction with LPS in three observation times resulted in similar results with the highest IL-6 level (1020.14%). IL-6 level was decreased significantly at 1 and 3 h after ketamine induction, with the lowest level (875.02%). This result suggested the inhibitory effect of ketamine on IL-6 level.

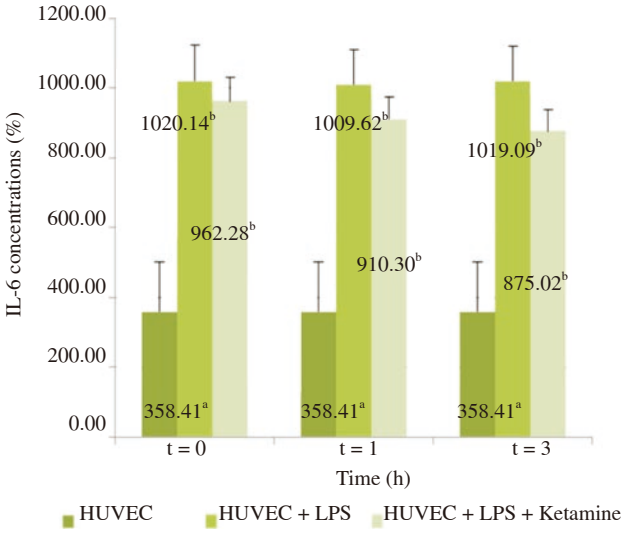

Figure 2. ELISA result of IL-6 measurement.
Different letters mean there is a significant difference among groups.

TNF-α measurement showed a similar result with IL-6 (Figure 3). The level of TNF-α was significantly elevated after LPS induction. The highest level of TNF-α level was shown at 1 h of incubation (517.55%). Ketamine induction decreased the expression of TNF-α level in all observation times. There was a slight increase in TNF-α level at every observation time in cultures induced by ketamine. The lowest level of TNF-α after ketamine induction (260.27%) was obtained in the first observation time.

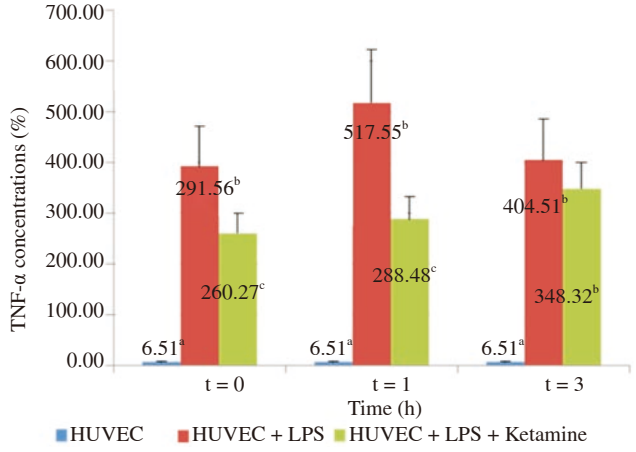

Figure 3. ELISA result of TNF-α measurement.
Different letters mean there is a significant difference among groups.

4. Discussion

The levels of all pro-inflammatory cytokines in this research were elevated from the beginning of LPS induction. These results were suggested to be the effects of co-culturing with monocytes.

Monocytes have an important role as inflammatory regulators and among the first component of the immune system activated in the sepsis process by expressing the pro-inflammatory cytokines[13]. Besides, in this research, LPS was used to induce the sepsis condition on culture. LPS is known as the major factor which could induce the occurrence of sepsis condition by increasing the transcription of genes encoding cytokines, chemokines, adhesion molecules, apoptosis factors, and many other inflammation mediators through monocytes[14,15].

In general, our result for NF-κB levels' measurement was similar to the previous study by Yu *et al*. in which the ketamine induction decreased the NF-κB level in rats sepsis model after 1 h of incubation[10]. The onset of ketamine is 2–10 min after induction then eliminated until it is below the therapeutic concentration[16-18].

Therefore, in our research, ketamine induction only lowered the NF-κB level in 1 h after induction. The activation of NF-κB will be followed by the increasing of IL-6 as one of the pro-inflammatory cytokines[19,20]. The inhibitory effect of ketamine was dose-dependent[21]. Although in other researches[22], the inhibitory effect of ketamine on IL-6 was obtained in high concentration, our research showed that 50 μmol/L of ketamine could decrease the IL-6 level. This result was suggested as the effect of direct treatment of ketamine after LPS induction. Finally, in the measurement of TNF-α, ketamine also has showed inhibitory effect. In the previous study, it was reported that ketamine could suppress the expression of TNF-α and pro-inflammatory cytokines, such as IL-6 induced by endotoxin in polymicrobial sepsis[23].

At least, in part of our *in vitro* results using HUVEC cultures to support the results of other researches was related with the effect of ketamine in suppressing LPS-induced inflammation. Generally, in our result ketamine suppressed the production of pro-inflammatory cytokines in all observation times. Further researches need to be carried out especially those related with various ketamine doses and its effect in genetic level.

Conflict of interest statement

We declare that we have no conflict of interest.

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