The Effect of *Escherichia coli* on Pro-Inflammatory Mediators Level and Kidney and Liver Function of Sepsis in *Rattus novergicus*

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Abstract

Sepsis is a life-threatening state of organ dysfunction caused by a deregulation of the body's response towards an infection. The purpose of this study was to determine the pro-inflammatory mediators' level as well as kidney and liver organ function on the *Rattus novergicus* sepsis model injected intraperitoneally (i.p.) with *E. coli*. This study used 20 male rats (*Rattus novergicus*, Wistar strain) which were divided into 2 groups: rats without *E. coli* injection and rats were i.p. with 105 CFU of E. coli. After six hours, the level of pro-inflammatory mediators (TNFα, Hs-CRP, PCT, and MDA), kidney function (urea, BUN, and creatinine), and liver function (SGPT, SGOT, and the total of bilirubin) were examined. The data obtained were analyzed using T-Test. This study concluded that intraperitoneal injection of *E. coli* increased pro-inflammatory mediator levels which include TNFα, Hs-CRP, PCT, MDA level. It also increased kidney function. Interestingly, the injection only increased bilirubin total levels in the liver but did not show improvement in the SGPT and SGOT. In summary, there was a significant increase in TNF-α pro-inflammatory mediators, procalcitonin (PCT), malondialdehyde (MDA), urea kidney function parameters and BUN among treatment groups. The hs-CRP, creatinine, and total bilirubin experienced a significant increase.

Keywords: Sepsis, Escherichia coli, pro-inflammatory mediators, Rattus novergicus, kidney, liver

Introduction

Sepsis is a life-threatening state of organ dysfunction caused by a deregulation of the body's response towards an infection. Where there is excessive body response due to infections that can occur in blood, urine, lungs, skin and other tissues[1,2,3]. Septic shock is defined as a state of sepsis where blood circulation and metabolism are abnormal. This condition can lead to significant death[4]. The symptoms and signs of sepsis vary widely. They are very nonspecific and usually give a systemic sign of pain. Sepsis is characterized by fever, mental disorder, hypotension, decreased urinary excretion, and thrombocytopenia. If the patients do not receive adequate therapy, sepsis will develop and turn into respiratory failure, kidney failure, coagulation abnormalities and even death.

Until now, sepsis is still the leading cause of death in the intensive care unit[5]. Globally, there are 27-30 million cases of sepsis worldwide and every approximately 9 million die (1 person dies every 3.5

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seconds)[6]. In developing countries, such as Indonesia, the mortality rate due to sepsis is two to three times higher than in developed countries, such as the USA. At the Pendidikan Hospital in Yogyakarta, 631 sepsis cases were found in 2007 with a mortality rate of 48.96%[8]. Various epidemiological researches stated that the incidence and mortality of sepsis are higher in men than women, although the cause of these findings is still unexplainable. In 2012-2013 at the Dr. Saiful Anwar General Hospital in Malang, Indonesia, a total of 1026 patients were diagnosed with sepsis and 788 of them died (76.8%). Whereas 168 sepsis patients were sent to the Intensive Care Unit (ICU) and 78 of them died (46.4%)[9].

Sepsis can be caused by a variety of microorganisms including viruses, bacteria, fungi, and protozoa[9]. The main cause of sepsis is lipopolysaccharide (LPS) exposure. Gram-negative bacteria are involved in 60-70% of sepsis epidemic, with some regional variations. Gram-positive bacteria also play a role in 30-50% of sepsis cases[10,11]. In recent years, murine sepsis models have become quite interesting topics of debate, especially those related to human diseases and the development of new biological therapy. Some researchers have reported an association between murine and human response to sepsis at the genomic level[12]. Some others argued that there are biological similarities among them. For example, a comparison between the complex human condition and homogeneous endotoxemia model in a single genetic strain of *Rattus novergicus*[13]. Among animal sepsis models, the *Rattus novergicus* is commonly used. It is due to the ease of handling, the availability of its genetic manipulation, and the relatively low cost[14]. Therefore, this study used sepsis rat injected intraperitoneally with 10^5 CFU of *E. coli*.

Materials and Methods

This study was approved by the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. This research uses an experimental post-test only control group design. The experimental animals used were male rats (*Rattus novergicus*), 7-9 weeks old, 150-170 grams body weight, had not undergone any treatment or had not received any chemicals, and were in a healthy condition. *Rattus novergicus* were divided into 2 groups: rats without E. coli injection and rats were i.p. with 10^5 CFU of *E. coli*. After six hours, the level of pro-inflammatory mediators (TNFα, Hs-CRP, PCT, and MDA), kidney function (urea, BUN, and creatinine), and liver function (SGPT, SGOT, and the total of bilirubin) were examined. The TNF-α, PCT, hs-CRP level was measured by using enzyme-linked immunosorbent assay (ELISA). Liver dysfunction was evaluated by measuring the total levels of bilirubin, aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT). Renal dysfunction was analyzed by measuring the levels of urea, nitrogen, and creatinine. All examinations were analyzed by the auto analyzer. The experimental data obtained were analyzed with the T-test.

Results and Discussion

The data shows that *E. coli* infection caused a significant increase in TNFα, PCT, MDA, urea, BUN (p <0.05) as well as the hs-CRP, creatinine and total bilirubin (p <0.01). Whereas the SGOT and SGPT did not show significant improvement (p >0.05).

Table 1. The results of the level of pro-inflammatory mediators, liver, and kidney functions in rats.

Variables	Control (X±SD)	E. coli Infection (X±SD)
TNF-α	0.58±0.16a	0.84±0.02b*
PCT	0.76±0.21a	1.06±0.09b*
hs-CRP	0.72±0.24a	1.17±0.10b**
MDA	0.13±0.03a	0.53±0.23b*
Urea	15.90±3.50a	24.46±4.75b*
BUN	7.43±1.64a	11.44±2.22b*
Creatinine	0.30±0.07a	0.54±0.05b**
SGOT	66.40±18.04a	95.20±36.48a
SGPT	31.00±15.08a	42.40±15.34a
Total Bilirubin	0.24±0.05a	0.42±0.08b**

The increase in TNF-α and MDA level has been reported in previous research where CLP (caecal ligation puncture) sepsis rat model and *E. coli* i.p. injection sepsis rat model was able to significantly increase both levels[15]. The most commonly used parameter of sepsis in humans is hs-CRP which is an examination of acute-

phase proteins. This parameter is more sensitive than CRP and LED in showing acute inflammatory processes in humans and PCT to see the severity of sepsis. In this study, hs-CRP experienced a very significant increase in hs-CRP and a significant increase in PCT.

E. coli is generally the main causative agent of extraintestinal infections, such as neonatal meningitis, bacteremia, pyelonephritis, cystitis, prostatitis, and sepsis. Paradoxically, this microorganism is also a dominant facultative member of normal human gut microbiota. Adhesion of pathogenic bacteria to host cells is the first step in establishing infection. Further events include tissue colonization and, in certain cases, cellular invasion followed by intracellular multiplication or persistence. The adhesion process begins when the surface structure, known as an adhesin, binds to their specific ligands, receptor host cells or extracellular matrix proteins[16,17]. Many of the previous sepsis-*E. coli* study showed changes in pro-inflammatory cytokines. This study showed changes in kidney function, where there is a significant increase in urea, BUN and creatinine level along with changes in liver function that represented by a significant increase in total bilirubin. However, the SGPT and SGOT levels did not change. This was due to the fact that an increase in SGPT and SGOT enzymes usually occurred after hepatocyte cell damage. The increase in liver enzymes occurred when there was inflammation in the liver after more than 24 hours[18].

In the *E. coli* group and control group, both showed a significant increase in TNF-α pro-inflammatory mediators, PCT, MDA, urea, and BUN levels. There was also a very significant increase in hs-CRP level, creatinine level, and bilirubin total level. Nevertheless, neither SGPT nor SGOT showed any improvements. *E. coli* i.p. injection can be used as one of the options to create a sepsis rat model which was easier to do and did not hurt experimental animals compared to the CLP method.

Conclusion

In summary, there was a significant increase in TNF-α pro-inflammatory mediators, procalcitonin (PCT), malondialdehyde (MDA), urea kidney function parameters and BUN among treatment groups. The hs-CRP, creatinine, and total bilirubin experienced a significant increase.

Conflict of Interest : The author declare that they have no conflict of interest.

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