Therapeutic Effect of Statins and LPS Antibody on Proinflammatory Mediator and Biochemical Markers of Sepsis Rat Model Induced by *E. coli*

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Abstract

This study aims to determine the effect of statins and LPS antibodies administration combined with *E. coli* intraperitoneal injection toward proinflammatory mediators (TNF-α, hs-CRP, PCT, and MDA) urea, BUN, and creatinine) and liver function (SGPT, SGOT, and total bilirubin) in sepsis rats. This study used 30 male white rats (*Rattus novergicus* strain Wistar) divided into 6 groups induced by *E. coli* bacteria combined with statins, LPS antibody (Ab-LPS), and a combination of statins+Ab-LPS. Examination of proinflammatory mediators (TNF-α, hs-CRP, PCT, and MDA) was carried out by the ELISA method through the examination of biochemical markers namely renal function (urea, BUN, and creatinine) and liver function (SGPT, SGOT, and total bilirubin) were analyzed by using the autoanalyzer. The data obtained were statistically analyzed using the T-test. The result shows that *E. coli*+statins gave significant changes on the MDA level at 0^{th} hour administration and TNF-α and hs-CRP at 3^{rd} hour administration. The *E. coli*+Ab-LPS treatment showed significant changes at the 0^{th} hour administration for MDA, creatinine, and total bilirubin and at the 3^{rd} hour administration for MDA. The *E. coli*+statins+Ab-LPS treatment showed significant changes in MDA, creatinine, and total bilirubin at 0^{th} hour administration and on creatinine and total bilirubin at 3^{rd} hour administration.

***Keywords: a**ntibody, LPS, E. coli, proinflammatory mediator, biochemistry marker, statins*

Introduction

Sepsis is a clinical syndrome that causes body excessive response to infection. Sepsis is also followed by a doubling response of the host to the infection that will lead to dysregulations of the host response[1,2]. Sepsis is characterized by fever, mental disorder, hypotension, decreased urinary excretion, and thrombocytopenia. Up to now, sepsis is still the main cause of death in the intensive care unit[3].

Sepsis was caused by various microorganisms including viruses, bacteria, fungi, and protozoa4. Gram-positive bacteria, such as *E. coli*, also plays a role in 30-50% of sepsis cases[5,6]. The main cause of sepsis is lipopolysaccharide (LPS) exposure. LPS is a proinflammatory endotoxin[7]. Elimination of natural killer cells (NK) and T cell activation can also be used as a sepsis therapy[4,8]. Anti-LPS antibodies were combined with statins. Statins acted as anti-inflammatory compounds to inhibit the expression of TNF-α and MCP-

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1 induced by LPS as well as inhibit the activation of NF-kB and AP-19. Side effects of statins administration are myopathy, a higher chance of diabetes, and increased transaminases serum[10]. Statins are usually prescribed with aspirin to prevent and treat cardiovascular disease by decreasing lipid serum levels[11,12].

In this study, we created sepsis rats model by inducing of *E. coli* intraperitoneally to determine changes in proinflammatory mediators (TNF-α, hs-CRP, PCT, and MDA) and biochemical markers on the kidney (urea, BUN, and creatinine) and liver (SGPT, SGOT, and total bilirubin).

Materials and Method

Ethical Clearance: All procedures involving animal care in a laboratory were approved by the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

Study Design

The research method used was an experimental laboratory using a post-test only control group design. The experimental animals used were male white rats, 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 (actively moves and no fall out feather). Thirty male white rats (*Rattus novergicus* strain Wistar) were divided into 6 groups. All groups were treated with E. coli bacterial induction. Then statins, Ab-LPS, and statins+Ab-LPS were added at 0th hour and 3rd hour respectively and were examined after 6 hours. The tools used in this study were mouse cages, water baths, stirrers, laminar airflow, ELISA readers, spectrophotometry, test tube rack, test tubes, micropipettes, intragastric feeding tube, and analytical balance. Examination of proinflammatory mediators (TNF-α, hs-CRP, PCT, and MDA) was performed using the ELISA method. The analyzes on kidney function (urea, BUN, and creatinine) and liver function (SGPT, SGOT, and total bilirubin) were done by using the autoanalyzer.

Data Analysis

All data were analyzed by using a T-test. The test was performed using SPSS Statistic 18 Software (IBM Corporation, USA).

Results

The effect of statins, Ab-LPS, and statins+Ab-LPS combination at 0th and 3rd hour toward proinflammatory mediators (TNF-α, hs-CRP, PCT, and MDA) and biochemical markers in the kidney (urea, BUN, and creatinine) and liver (SGPT, SGOT, and total bilirubin) is presented on Table 1.

Table 1. The effect of statins, Ab-LPS, and statins+Ab-LPS combination at 0th hour and 3rd hour toward proinflammatory mediators and biochemical markers in sepsis rats model induced by *E. coli*.

Variables	E. coli+Statins (X±SD)		E. coli+Ab-LPS (X±SD)		E. coli+Statins+Ab-LPS (X±SD)	
	0th-hour treatment	3rd-hour treatment	0th-hour treatment	3rd-hour treatment	0th-hour treatment	3rd-hour treatment
TNF-α	0.75±0.12ab	0.63±0.12a	0.66±0.15ab	0.76±0.06ab	0.81±0.16ab	0.76±0.07ab
hs-CRP	0.96±0.15ab	0.72±0.23a	0.96±0.24bc	1.21±0.15c	1.00±0.17ab	0.83±0.16ab
PCT	0.94±0.18a	0.93±0.25ab	0.90±0.34a	0.83±0.16ab	1.01±0.32a	1.05±0.14ab
MDA	0.17±0.07a	0.16±0.06a	0.23±0.11a	0.25±0.18a	0.17±0.24a	0.30±0.08ab
Urea	24.43±0.58b	21.28±4.23ab	19.96±7.04a	24.14±5.66b	17.56±3.37ab	20.48±2.58ab
BUN	11.53±0.25b	10.18±1.97ab	9.35±3.30ab	11.27±2.64b	8.26±1.64ab	9.57±1.21ab
Creatinine	0.46±0.05b	0.30±0.10a	0.34±0.13a	0.46±0.05b	0.30±0.07a	0.38±0.06a
SGPT	39.40±12.22	33.20±12.99	28.00±6.96	38.20±12.13	38.20±12.13	31.00±15.08
SGOT	90.00±28.83	69.15±31.72	67.80±18.29	87.40±36.26	66.40±36.56	79.00±22.65
Bilirubin	0.36±0.11a	0.30±0.07ab	0.26±0.05a	0.36±0.09ab	0.30±0.12a	0.26±0.09a

Discussion

E. coli is gram-negative, rod-shaped bacterium that generally causes extraintestinal infections, such as neonatal meningitis, bacteremia, pyelonephritis, cystitis, prostatitis, and sepsis. Ironically, this microorganism is also a dominant facultative member of normal human gut microbiota. Adhesion is the first step of pathogenic bacteria to infect its host cell. Furthermore, the 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[13].

The results showed that at the 0th and 3rd hour of administration the proinflammatory mediator TNF-α on *E. coli*+statins group, *E. coli*+Ab-LPS group, and *E. coli*+Statins+Ab-LPS group did not differ significantly (p>0.05). Statinss administration at the 3rd hour gave a significant change in TNF-α. This was due to excessive amounts of LPS so that it was able to induce a high level of proinflammatory cytokines (TNF-α, IL-6) production in blood circulation. This condition has caused acute inflammation, tissue apoptosis, lead to endotoxic organ injury[14,15,16].

The administration of statinss at the 3rd hour gave a significant change in hs-CRP, while at the 0th hour there was no significant change. Hs-CRP has been used to help the diagnosis or refers to some therapies[17]. Significant changes occurred in PCT both at the 0th and 3rd hours. PCT is a group of peptides consisting of 116 amino acids and calcitonin precursors. The protein is synthesized by parafollicular cells, lung neuroendocrine cells, and few amounts were located in the small intestine[18]. In this study, statins and Ab-LPS administration gave significant changes to MDA at 0th hour and 3rd hour, whereas statins+Ab-LPS combination showed significant changes in MDA at 0th hour administration. MDA levels play a role as a marker of oxidative damage that occurs in polyunsaturated fatty acids (PUFA) due to the induction of *E. coli*. MDA is an aldehyde molecule that results from the formation of free radicals in polyunsaturated fatty acids. The measurement of MDA levels was performed to indicate oxidative damage[19,20,21].

Statins treatment did not give any significant changes in biochemical markers of the kidney (urea and BUN), in either the 0th hour or the 3rd hour administration. However, there was a significant change in creatinine in both the 0th and 3rd hour. The administration of Ab-LPS did not show significant changes in urea and BUN in both the 0th and 3rd hours. The administration of statins+Ab-LPS combination did not change the urea and BUN significantly. It was due to many factors that influence urea and BUN increase so that the levels of urea or BUN are easy to change sepsis condition or before sepsis occurs.

In this study, the administration of Ab-LPS and combination of statins+Ab-LPS showed a significant change in creatinine at the 0th hour administration. Whereas statins administration and statins+Ab-LPS combination showed significant changes in creatinine at the 3rd hour administration. Statins+Ab-LPS administration did not give significant changes on SGPT and SGOT, however, it gave significant changes on bilirubin at 0th and 3rd hour administration. In this study, there was no significant difference between SGPT and SGOT in terms of examination time (p>0.05). The increase in SGPT and SGOT enzymes occurs after hepatocyte damages due to inflammation caused by *E. coli*. The increases of SGPT and SGOT occur after more than 24 hours of *E. coli* infection[22]. Study on using statinss as therapeutic agents in sepsis cases has been able to prove that statinss can increase survival, ability, function, and protection of organs in sepsis rat induced by LPS or caused by CLP[23,24,25,26].

Conclusion

In sum, this study found that the administration of *E. coli*+statins at 0th hour gave significant changes to MDA levels and TNF-α and hs-CRP at 3rd hour administration. The *E. coli*+Ab-LPS treatment showed significant changes in MDA, creatinine, and total bilirubin levels at 0th hour administration and only MDA at 3rd hour administration. The *E. coli*+statins+Ab-LPS treatment showed significant changes in MDA, creatinine, and total bilirubin levels at 0th hour administration and creatinine and total bilirubin at 3rd hour administration.

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

Source of Funding: This study funded by the the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia.

Acknowledgement: We thank EJA – Professional Translation Services for editing the manuscript.

Ethical Approval: This study was approved by the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

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