The correlation between caspase-9 and caspase-3 on sepsis case: an experimental study on the Balb/C Mice induced by lipopolysaccharide

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ABSTRACT

Introduction: Sepsis mortality rate in Indonesia reaches over 50%, hence, acknowledgement of its pathophysiology need to be elucidated, including the pathway of apoptotic. The objective of this study was to describe the lipopolysaccharide (LPS)-induced sepsis in which triggers intrinsic apoptotic pathways in terms of the expression of caspase-9 (Cas9) and caspase-3 (Cas3).

Method: By experimental with time series randomized post-test-only control group design, 48 Balb/C mice divided into 2 groups (control and treatment). Each group was injected intra-peritoneally with saline 250 µL/mice and saline 250 µL + 0.1 mg E. coli LPS/mice. Each group divided into 4 termination time (12th, 24th, 36th and 48th hours), examined by immunohistochemistry (IHC) for Cas9 and Cas3 expression, and analyzed by mean difference test (Mann-Whitney and t test) and correlation test (Spearman’s test).

Results: The LPS-induced sepsis in the treatment group revealed increasing number of caspase-9 compared to control group (2.34±0.24 vs 0.82±0.08; p<0.001). There were increasing trends in the treatment group compared to control group includes for: all groups Cas3 (1.54±0.10 vs 0.48±0.05; p<0.001), t12 Cas3 (3.25±0.22 vs 0.50±0.06; p<0.001), t24 Cas3 (1.37±0.27 vs 0.45±0.05; p=0.002), and t36 Cas3 (1.03±0.20 vs 0.52±0.22; p=0.002). Instead, there were no difference between treatment group and control group for t48 Cas3 (0.50±0.14 vs 0.43±0.10; p=0.485). There was decreasing pattern on serial time 12, 24, 36 and 48 hours for Cas9 (p<0.001) and Cas3 (p=0.002). There was a correlation between Cas9-Cas3 (r²=0.835; p=0.001).

Conclusion: The LPS-induced sepsis caused an increase of caspase-9 and caspase-3, strongly correlated with decreasing pattern on time serial.

Keywords: Caspase-9, caspase-3, lipopolysaccharide


INTRODUCTION

The high mortality rates of sepsis, includes 30% of severe sepsis, 60% of septic shock in America, and more than 50% of sepsis in Indonesia, indicates that an increase in the efforts to treat sepsis is urgently needed. The understanding of the pathophysiology of sepsis in bio-molecular aspects will provide the basis and guidance for the better subsequent treatment stages.

Sepsis begins as the consequence of pathogen port de entry, including gram-negative bacteria, where the outer membrane contains endotoxin lipopolysaccharide (LPS); LPS plays an important role in the constellation of sepsis. Pathogen virulence and excessive immune responses will cause such damage, including the damage to mitochondria, failing intra-cell oxidative energy process and cell death. Apoptosis plays an important role in the pathogenesis of sepsis. Hence further acknowledgement of LPS, bio-molecular and apoptotic pathways are needed to understand the pathophysiology of sepsis and stand as the basis for future treatment of sepsis.

Apoptotic pathways can be triggered by 2 mechanisms. The first pathway is mediated by extrinsic receptors. The second pathway is mediated by mitochondrial damage that modulates the intrinsic pathway by releasing mediators activating caspase-9 (Cas-9). These two pathways will produce caspase cascades which in turns results in apoptosis. The presence of dATP might cause cas-9 to be directly activated by apaf-1 and cytochrome c. Subsequently, the active form cas-9 will modulate the caspase 3 (cas-3) causing the apoptotic apparatus taking part in DNA fragmentation and cell death. Thus, this study aimed to prove that sepsis induced by LPS causes an increase in cas-9 and cas-3; to determine the cas-9 and cas-3 expression pattern in the time serials after exposure to LPS; to determine the relationship between cas-9 and cas-3.

MATERIAL AND METHODS

Experimental time series randomized post test-only control group design in 48 Balb/C mice was used. The samples were divided into 2 equal groups. The control group samples were injected with sterile saline 250 µL/mice, whereas the treatment group was injected with 0.1 mg of LPS E.coli in 250 µL saline/mice. Each group was divided into 4 subgroups according to termination time (each group with 6 mice) and terminated at 12, 24, 36 and 48 hours.
were terminated by neck dislocation. Intestinal tissue was taken and performed the assays for cas-9 expression and cas-3 with immunohistochemistry (IHC).

RESULTS

Our study showed in descriptive data of both cas-9 and cas-3 expression by 2 reading device (device 1 and device 2) based on the treatment and control groups. The distribution of data from device 1 and device 2 on cas-9 and cas-3 expression were not normally distributed (p < 0.05 with the Shapiro-Wilk test). In terms of the correlation between device 1 and device 2 on cas-9 and cas-3 scores, cas-9 expressions across groups were strongly correlated (r = 0.841) and so do the expression of cas-3 of the whole group (r = 0.865). These results showed a parallel conjunction between the device readers. The data used for subsequent statistical processing were the average of the two devices used.

A cas-9 expression among the treatment and control groups

The distribution of cas-9 expression data was normal for the 12th, 24th, 36th and 48th termination groups with p > 0.05 both in the treatment and control groups, therefore, the mean difference test was done by parametric/t test. Meanwhile, caspase-9 expression data for the 36th termination group was not normally distributed, with p < 0.05 using the Shapiro-Wilk test, hence, the mean difference test used non-parametric/Mann Whitney tests, as shown in Table 1.

The treatment group exhibited an increase in cas-9 expression compared to the control group in all time of terminations at 12, 24, 36 and 48 hours; all groups statistically significant (see Table 1 and Figure 2). Instead, caspase-9 expression patterns in serial termination times at 12, 24, 36 and 48 hours were significantly decreased (p < 0.001).

A caspase-3 expression among the treatment and control groups

The data of cas-3 for 12th and 36th termination groups were normally distributed (p > 0.05) so that the mean difference tests were done by parametric/t test. In the other hand, the distribution of 24th and 36th termination group for cas-3 expression data were not normal (p < 0.05), therefore, mean difference test used was non-parametric/Mann-Whitney tests, as shown in Table 2.

The treatment group experienced an increase in caspase-3 expression compared to controls in all groups of termination at 12th, 24th, and 36th hours; all groups differs significantly statistically. At 48th hour termination of cas-3 expressions, the data did not differ significantly between treatment and control groups, as shown in Table 2 and Figure 4.

Correlation test has been done between caspase-9 and caspase-3 (Table 3 and Table 4). There were a very strong positive correlation between caspase-9 and
caspase-3 among all groups (rs = 0.835 ** / p = 0.001), at the 24th hour termination group (rs = 0.846 **/p = 0.001) and at 36th hours termination (rs = 0.912 **/p = 0.000). A strong positive correlation was also found between caspase-9 and caspase-3 at the 12th hours termination group (rs = 0.679 */p = 0.015). These phenomenon might indicate that sepsis induced by LPS mediating apoptosis via intrinsic pathway. The positive correlation was moderate between caspase-9 and caspase-3 at the 48th hour termination group (rs = 0.377 /p = 0.227) because LPS exposure disappeared within the first 48 hours yet did not occur again (shown in Table 2 and Table 3).

**DISCUSSION**

The increase in caspase-9 expression in the treatment group might happen because of the activation of the apoptotic process through intrinsic pathways in the sepsis group. The mean caspase-9 expression in the treatment group was significantly higher compared to the control group. There was an increase in caspase-3 expression in Balb/C sepsis mice induced by LPS. The increase in caspase-3 expression was a sign of an increase in ongoing apoptotic process in sepsis case. The mean caspase-3 expression was significantly higher than the control group.

Caspase-9 and caspase-3 expressions showed a decreasing pattern in time serial of 12th, 24th, 36th and 48th hours. This phenomenon was in accordance with clinical symptoms that improved from the 3rd day. In addition, there was a significant correlation between caspase-9 and caspase-3 expression (rs = 0.835; p = 0.001), as the sign of apoptotic activation through the intrinsic pathway.

Sepsis is a life-threatening illness that occurs due to an abnormal host immune network which extends through the initial widespread and overwhelming inflammation. During the initial phase of sepsis, a vigorous induction of the innate immune system can cause exaggerated production of pro-inflammatory cytokines, chemokines, and other inflammatory mediators. All processes will lead to Systemic Inflammatory Response Syndrome (SIRS) whereas caspases also have a pivotal role in this process.

The caspases are a family of cell proteases that exist as zymogens within cells and can be activated by either autocatalytic activation or other proteases. Role of caspases in sepsis is related to the apoptotic signaling pathway. Apoptosis is classically triggered via two signaling pathways, the intrinsic pathway (also known as the mitochondrial pathway) and the extrinsic pathway (also known as the death receptor pathway). The extrinsic pathway is triggered when death ligands (for example, FasL, TNFα, AproL, and TRAIL) bind to their respective cell surface death receptors (Fas, TNFR1, DR3, DR4, and DR5). Increased level of Cas-3 and Cas-9 was related to the improves survival and less T and B-Cell apoptosis. Less of apoptotic response initiates...
both cell work to release many anti-inflammatory cytokines that can interfere the SIRS response due to LPS induction.\(^{12,13}\) A previous studies found extensive evidences for apoptosis of lymphocytes and gastrointestinal epithelial cells during sepsis.\(^{14}\) More importantly, the evidence from several animal models strongly suggests that inhibition of apoptosis in both sepsis and acute lung injury/acute respiratory distress syndrome improves survival.\(^{5}\) In addition, a decrease level of both caspases (Caspases-3 and Caspases-9) based on time interval in our study suggested for resolution phase which is in accordance with the systemic response to prevent further pro-inflammatory cytokine related with sepsis.

### CONCLUSIONS

LPS-induced sepsis in Balb/C mice caused in increasing caspase-9 and caspase-3 expression also decreased caspase-9 and caspase-3 in time serials at 12, 24, 36 and 48 hours after exposure to LPS. There was a strong positive correlation between caspase-9 and caspase-3 expression. Further research is needed based on the apoptotic stage to deepen understanding of the impact of sepsis due to lipopolysaccharide exposure, particularly in the extrinsic pathway.

### CONFLICT OF INTEREST

There is no competing interest regarding manuscript.

### ETHICAL CLEARANCE

Ethical approval has been obtained prior to study conducted from ethics committee of Faculty of Medicine and Health Science, University of Jambi.

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AUTHOR CONTRIBUTION

The authors are equally contributed to the study from manuscript preparation, data analysis, until current report of study.

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