ABSTRACT

Objective: Heat shock protein 70 (HSP70) decreases Cyt expression, Bax, and Caspase 3 in apoptosis multiple organ dysfunction syndrome (MODS), thus inhibiting death. This study aimed to analyze the efficacy of HSP70 200 µg/KgBB/ip to decrease Cyt c, Bax, and Caspase 3 expression, to reduce mortality, and to increase survival rate, in the MOD alveolar lung epithelial of 78-h sepsis model.

Methods: This was a post-test only quasi-experiment conducted at Inter-University Central Laboratory of Gadjah Mada University, Yogyakarta, and the Anatomy Pathology Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta. The study used a type of Bab/c mice, male, aged of 6–8 weeks, body weight of 25–33 g. Sepsis induction used LIG SIGMA L2880-10MG Lot #025M4040V from Escherichia coli 055:B5 purified by phenol extraction. Medication to reduce mortality used HSP70 Lot #L16020515 and then continued with 400× immunohistochemistry (IHC) examination. A sample of 30 mice were divided into three groups: (1) Control group without 78 h treatment, (2) lipopolysaccharide (LPS) group with a dose of 0.25 mg/kgBW/ip 78 h, and (3) HSP70 group with a dose of 200 µg/kgBW/ip after LPS injection 0.25 mg/kgBW/ip 78 h. The outcome variables included expression of Cyt, Bax, Caspase 3, and mortality in mice model with multiple organ dysfunction syndrome. The data were analyzed by Kruskal–Wallis and continued by Mann–Whitney U-test.

Results: Administration of HSP70 200 mg/KgBW/ip after LPS 0.25 mg/kgBW/ip significantly decreased Cyt expression (p=0.014), Bax (p=0.004), and Caspase 3 (p=0.015) in 78 h pulmonary alveolar cells, reduced mortality rate, and increased the number of survivors. Expressions of Cyt, Bax, and Caspase 3 of IHC 400× magnification had a near-normal image change.

Conclusion: There is a decrease of Cyt c, Bax, and Caspase 3 expression in the MOD alveolar lung epithelial cells of the 78-h sepsis model mice, a decrease of mortality rate, and an increase of survival rate, and the image of IHC is almost normal.

Keywords: Sepsis, Multiple organ dysfunction syndrome, Apoptosis, Heat shock protein 70.
caspase 9 and 3 [11]. Sharma and Maseon state that HSP70 can affect apoptosis through the interaction of complementary proteins and antapoptotic proteins BCL 2. Increased HSP70 protects cytotoxicity cells from apoptotic, radiation, and chemotherapeutic agents. HSP70 binds directly to apaf 1 protease and prevents apoptosis formation [19]. Results of Mazzei et al. show that HSP70 inhibits apoptosis and inflammation, repairs damaged proteins, prevents folded protein aggregation, and targets damaged proteins for degradation and cytoskeletal stabilization [20].

This study aimed to determine the effect of HSP70 200 mg/KgBW/i.p post-LPS 0.25 mg/KgBW/i.p on the expression of Cyt c, Bax, and Caspase 3 in 78 h of lung alveolar cells, mortality, and the number of survivors.

METHODS

This study used laboratory experimental research designed post-test only group. Maintenance and treatment were carried out at the Pau Laboratory of Gadjah Mada University, Yogyakarta, from 1 to April 21, 2017, and then continued with immunohistochemistry (IHC) examination at the Anatomy Pathology Laboratory, the Faculty of Medicine, Universitas Sebelas Maret, Surakarta. Subjects were mice of Balb/c strain, male, aged 6–8 weeks with a body weight of ±25–30 g. The drug used to cause sepsis was LPS dose of LD₅₀ 0.25 mg/KgBW from SIGMA L2880-10MG Lot #025M40404V LPS from Escherichia coli 055:B5. HSP70 Lot #16020515 drug was used to reduce the expression of Cyt c, Bax, and Caspase 3, to reduce mortality, and to increase survival rates. Ethical reviews for this study were provided by the research ethics committee of Dr. Moewardi Hospital, Surakarta, Central Java, Indonesia, number 377/IV/HREC/2017.

A sample of 30 mice was divided into three groups of 10 mice for each. P0: Normal control with sodium chloride. P1: The treatment group received 0.25 mg/KgBW/i.p. P2: The treatment group received HSP70 injection of 200 μg/KgBW/ip post-LPS 0.25 mg/KgBW/ip.

The obtained data were statistically analyzed by one-way ANOVA test of the SPSS for Windows Release 11.5, and p<0.05 was chosen as the minimum level of statistical significance. The one-way ANOVA test requirement is a numerical scale and the data distribution is normal and homogeneous. If the one-way ANOVA test showed a significant difference (p<0.05), the result would be proceeded with the LSD post hoc test. If the one-way ANOVA test requirement could not be fulfilled, the non-parametric alternative test of Kruskal–Wallis would be used. If the Kruskal–Wallis test showed significant differences (p<0.05), the post hoc test would be continued using the Mann–Whitney U-test.

RESULTS

There were a total of 30 mice of Balb/c strain, male, aged 6–8 weeks with a body weight of ±25–30 g. In the treatment group that received LPS injection of 0.25 mg/KgBW/ip, there was an increase in Cyt c expression (p=0.011), Bax (p=0.005), and Caspase 3 (p=0.011) while in the treatment group that received HSP70 of 200 μg/KgBW/ip injection after LPS 0.25 mg/KgBW/ip there was a decrease in Cyt c expression (p=0.014), Bax (p=0.004), and Caspase 3 (p=0.015) as shown in Table 1.

Whereas, the expression of Cyt c, Bax, and Caspase 3 expression in alveolar epithelial cells lung of 78-h sepsis can be seen in Diagram 1: (1) KN: Control group without treatment but only injected by 1 cc NaCl intraperitoneal, (2) treatment group that received 0.25 mg LPS injection/KgBW/ip to make sepsis, and (3) HSP70 group of 200 μg/KgBB/ip after LP 0.25 mg/KgBW/ip on the lung alveolar epithelium of 78-h MODS model sepsis can be seen in Diagram 1.

There was a significant increase in Cyt c expression (p=0.011), Bax (p=0.005), and Caspase 3 (p=0.011) in 78 h of pulmonary alveolar epithelial cells after LPS injection HSP70 administration significantly decreased Cyt c expression (p=0.014), Bax (p=0.004), and Caspase 3 (p=0.015) in 78 h of pulmonary alveolar epithelial cells after LPS injection (Fig. 1).

The results of IHC alveolar epithelial of 78-h sepsis at 400× magnification in normal control (NC) where no treatment was performed show a basically brownish blurry appearance and Cyt c, Bax, and Caspase 3 expression in a less bright brown color with a blurry base. In those who receive LPS injection 0.25 mg/KgBW/ip, the expression looks brighter, denser, and the base looks brighter compared to that of NC. In the HSP 200 mg/KgBW/ip injection, the expression appears to be a reduced brownish color and less dense and the base is less bright and almost the same as that of NC.

DISCUSSION

LPS injection of 0.25 mg/KgBW/ip was able to make sepsis in mice with increased expression of Cyt c, Bax, and Caspase 3. These results are almost in line with Guntur study on making sepsis with LPS 0.1 mg/Kg BW [1]. In contrast, the results of Markwart et al. show LPS injection of 9–11 mg/KgBW/ip injected once cause systemic inflammatory response syndrome and sepsis [21]. Zhao et al. suggested that low dose LPS ip injection of 5 mg/KgBW is used for studies of septis and organ injury while high doses of 15 mg/KgBW cause death [22]. Fodor et al. increased LPS injection of 3.5, 10 mg/KgBW/ip at 6 h resulting in a correlation in dose increase with the severity of hypoxemia [23].

At the injection of HSP70 200 μg/KgBPB/ip, there was a decrease in the expression of Cyt c, Bax, and Caspase 3. However, in the previous studies of HSP70, the dose was generally 266 ug/KgBB/ip to

Table 1: Results of Chi-square test using expression of Cyt c, Bax, and Caspase 3 with LPS injection of 0.25 mg/KgBW/ip to make sepsis and to those receiving HSP70 injection of 200 μg/KgBW/ip at alveolar lung epithelium of 78-h MODS sepsis model

<table>
<thead>
<tr>
<th>Organ</th>
<th>LPS Cyt c</th>
<th>LPS Bax</th>
<th>LPS Caspase 3</th>
<th>HSP70 Cyt c</th>
<th>HSP70 Bax</th>
<th>HSP70 Caspase 3</th>
</tr>
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<tbody>
<tr>
<td>Pulmonary alveolar epithelium of 78 h</td>
<td>p=0.011</td>
<td>p=0.005</td>
<td>p=0.011</td>
<td>p=0.014</td>
<td>p=0.004</td>
<td>p=0.015</td>
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Pulmonary alveolar epithelium of 78 h: LPS has a significant increase in Cyt c, Bax, and Caspase 3. HSP 70 has a significant decrease in Cyt c, Bax, and Caspase 3. LPS: Lipopolysaccharide, HSP70: Heat shock protein 70, MODS: Multiple organ dysfunction syndrome

Diagram 1: Results of Cyt c, Bax, and Caspase 3 expressions in alveolar epithelial cells of 78-h sepsis. Range magnitude: Value 1: Range 1–30%, Value 2: Range 31–70%, Value 3: Range 71–100%
CONFLICTS OF INTEREST
The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES