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Experimental Research

The effect of glutathione as adjuvant therapy on levels of TNF-α and IL-10 in wistar rat peritonitis model

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ABSTRACT

Background: Peritonitis is the second most common cause of severe sepsis that associated with a significant mortality rate. Due to a large gap of newer antibiotics innovation and antibiotic resistance emergence, the use of antioxidant has a possible alternative as adjuvant therapy in peritonitis management. It has been studied that glutathione as an alternative in the development of new anti-inflammatory effect. Thus, the aim of this study was to evaluate the levels of TNF-α and IL-10 after glutathione administration as adjuvant therapy in rat peritonitis model.

Materials and methods: Male wistar rats were divided into four groups (n = 6 per group), Group 1: control group (C), Group 2: peritonitis group (P), Group 3: peritonitis + Ceftriaxone group (P + Cef), Group 4: peritonitis + Ceftriaxone + Glutathione group (P + Cef + Glu). Twenty-four hours after peritonitis induction, the blood samples were taken to evaluate TNF-α and IL-10 levels.

Results: There was a significantly increase of mean TNF-α level in group 2 (P) 473,86 ± 388,99 pg/ml (p value 0,00) and significantly decrease of mean TNF-α level after glutathione injection in group 4 (P + Cef + Glu) (p value 0,02). No significant changes in IL-10 levels in rats peritonitis model.

Conclusions: Glutathione supplementation is significantly decrease the mean level of TNF-α in rats induced peritonitis, however there is no difference compare to antibiotic only. Moreover, there no significant changes level of IL-10 in rats induced peritonitis after glutathione injection.

1. Introduction

Peritonitis is the second most common cause of severe and associated with a significant mortality rate [1], with overall mortality was 9,2% (416/4533) in 132 medical institutions worldwide during 4-month period [2]. One of the principle management of peritonitis is the use of empiric antibiotics [1]. However, due to a large gap of newer antibiotics innovation[3] and antibiotic resistance emergence [4], the use of antioxidant has a possible alternative as adjuvant therapy in peritonitis management.

Glutathione is an antioxidant which known to exhibit numerous health benefits such as anti-ageing, anti-apoptotic and immune-stimulatory effects. It also been studied as new anti-inflammatory agents [5,6]. Previous study showed that with antioxidant supplementation significantly increase bacterial load in mice carrying *E. coli* induced acute bacterial peritonitis. However decreased number of macrophages, B-cells and dendritic cells at the primary site of infection [7]. On the other studies showed that several antioxidant agents have anti-inflammatory effects by decreased the cytokines [8,9]. Some suggested that the antioxidant inhibit the activation of NF-κB *(Nuclear Factor Kappa-B)* [10,11] followed by inhibition of cytokines release such as TNF-α and IL-10.

TNF-α is a key pro-inflammatory cytokine after inflammatory stimulation that can activated cascade reactions during inflammation and directly reflects the severity of an inflammatory response [12]. IL-10 is anti-inflammatory cytokine that can inhibit the release of pro-inflammatory cytokines [13], has been found increased in peritoneal exudates as a counterbalance response to local inflammatory

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production of cytokines [14]. However, a prolonged increase in IL-10 may suppress immune response and aggravate the severity of disease. Therefore, it's important to maintain the ratio of IL-10/TNF-α in proper level 1,3–1,9 [15].

Several reports remains pro- and contra- about the antioxidant supplementation that became trigger this study was conducted. The aim of this study was to evaluate the levels of TNF-α and IL-10 after glutathione administration as adjuvant therapy in rat peritonitis model.

2. Methods

2.1. Experimental animals

Male Wistar rats weighing 250 ± 50 g, aged 6–8 weeks. The rats were housed at 28,0 ± 2,0 °C room temperature with 12 h light/dark cycle and were fed rodent chow and water *ad libitum*. The Wistar rats underwent 7 days of acclimatization before experiment was begun. The experiment was approved by Research and Ethics Committee of Faculty of Medicine Diponegoro University, Indonesia (protocol number: 29/EC/H/FK-UNDIP/V/2020) and fully compliant with ARRIVE criteria [16].

2.2. Peritonitis induction

Bacterial suspension 1,5 ml containing 10^7 CFU/ ml *Escherichia coli* was administered intraperitoneally to rats.

2.3. Animal groups and study design

This is a randomized post-test only study with control group. Twenty-four rats were divided into four groups (n = 6 per group) as follows: Group 1 is control group (C). Group 2, peritonitis group (P), 1,5 ml × 10^7 CFU/ ml *E. coli* suspension was injected intraperitoneally. Group 3, peritonitis + Ceftriaxone group (P + Cef), which intravenous Ceftriaxone injection (186 mg/ kg body weight) was made 1 h after the injection of *E. coli* suspension. Group 4, peritonitis + Ceftriaxone + Glutathione group (P + Cef + Glu), which intravenous Ceftriaxone injection (186mg/ kg body weight) and intravenous Glutathione injection (250 mg/ kg body weight) was made 1 h after the injection of *E.coli* suspension. The dose of Ceftriaxone and Glutathione was adjusted or converted regarding the pharmacokinetic of the drugs for rats [17].

2.4. Cytokines analysis

Twenty-four hours after peritonitis induction, the blood samples were taken from all rats. TNF-α and IL-10 from blood samples were studied through commercial ELISA kit, following the instructions supplied by the manufacturer (*Koma Biotech INC.*,), lot number were 48225 and 08233 respectively, at GAKI Laboratorium Diponegoro University.

2.5. Statistical analysis

All data were evaluated using SPSS 23.0 for Mac Software. The results were expressed as mean ± standard deviation. *Saphiro-Wilk* test was using for data normality test. The data was compared using *One-way ANOVA test, Kruskal-Wallis* test following *Mann-Whitney* test. Statistical significance was defined as $p < 0,05$.

3. Results

3.1. Normality test

The *Saphiro-Wilk* normality test revealed that the majority of the basic variables in TNF-α level and IL-10 level did not have a normal distribution ($p < 0,05$). IL-10/TNF-α ratio data showed a normal distribution with $p > 0.05$.

3.2. TNF-α level

The non-parametric *Kruskal-Wallis* test showed there was a significantly difference in TNF-α level in all rats (p value 0,02) (Table 1.). *Mann-Whitney* test showed there was a significant difference level between group 1–2 (p value 0,00) and group 2–4 (p value 0,02) (Table 2). We found significant increase of TNF-α mean level in group 2 (peritonitis group) 473,86 ± 388,99 pg/ml, which the highest among all groups (Fig. 1.). Furthermore, there was a significant decrease of TNF-α mean level in group that received Gluthatione as adjuvant therapy (Group 4: Peritonitis + Ceftriaxone + Glutathione) compare to group 2 (peritonitis group).

3.3. IL-10 level

The non-parametric *Kruskal-Wallis* test was used to compare IL-10 levels in all groups, which showed there was no significant difference of IL-10 mean levels in all samples (p value 0,75) (Table 1.). However, there was a similar trend with TNF-α levels (Fig. 2.), that there was an increase of mean level of IL-10 in group 2 (peritonitis group) 39,15 ± 30,94 pg/ml. The same pattern was observed in group 3 (Peritonitis + Ceftriaxone) and group 4 (Peritonitis + Ceftriaxone + Glutathione), which decrease to 20,31 ± 11,92 pg/ml and 21,48 ± 4,09 pg/ml, respectively.

3.4. IL-10/TNF-α ratio

Our result revealed that overall IL-10/TNF-α ratio in all groups were fell below the normal threshold (1,3-1,9) (Table 3). The *Saphiro-Wilk* normality test revealed that the IL-10/TNF-α ratios have a normal distribution. *One-way ANOVA* test showed that there's no significant difference of IL-10/TNF-α ratio in all groups ($p > 0,05$).

4. Discussion

In our study revealed that there was a significant difference of TNF-α level in all groups (p value 0,02) (Table 1.). *Mann-Whitney* test showed there was a significantly difference level of TNF-α between group 1–2 (p value 0,00). It's similar to the study that was conducted by Goswami et al. that levels of IL-1β, IL-6 and TNF-α were significant increase in mice induced peritonitis group compared to the control group (7). Increased the levels of proinflammatory cytokines such as TNF-α in animal experiment induced by peritonitis indicate the activation of the immune mechanism in fight against infection. Therefore, early detection of TNF-α is great to evaluate the severity of intraabdominal infection [18].

Table 1
Data distribution on TNF-α and IL-10 levels in all groups (pg/ml).

Parameter	Group	Min	Max	Mean ± SD	Median	p
TNF-α	1 (C)	10,63	80,29	37,79 ± 26,50	35,8	0,02*
	2 (P)	133,30	1179,30	473,86 ± 388,99	371,27	
	3 (P + Cef)	8,03	670,72	204,66 ± 281,70	47,20	
	4(P + Cef + Glu)	26,35	498,46	137,43 ± 180,38	68,69	
IL-10	1 (C)	6,81	29,29	18,44 ± 10,00	18,87	0,75*
	2 (P)	14,97	86,55	39,15 ± 30,94	22,57	
	3 (P + Cef)	8,79	36,19	20,31 ± 11,92	18,91	
	4(P + Cef + Glu)	17,11	29,29	21,48 ± 4,09	20,36	

**Kruskal-Wallis test* ($p < 0,05$).

Table 2
Difference analysis in TNF-α levels.

Group	1(C)	2 (P)	3(P + Cef)	4(P + Cef + Glu)
1(C)	–	0,00*	0,52	0,10
2 (P)	–	–	0,10	0,02*
3 (P + Cef)	–	–	–	0,63

**Mann-Whitney test* ($p < 0,05$).

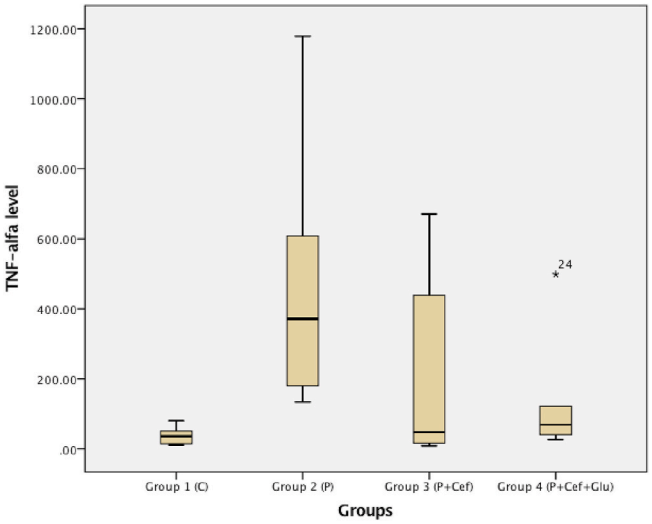

Fig. 1. Box-plot of TNF-α levels.

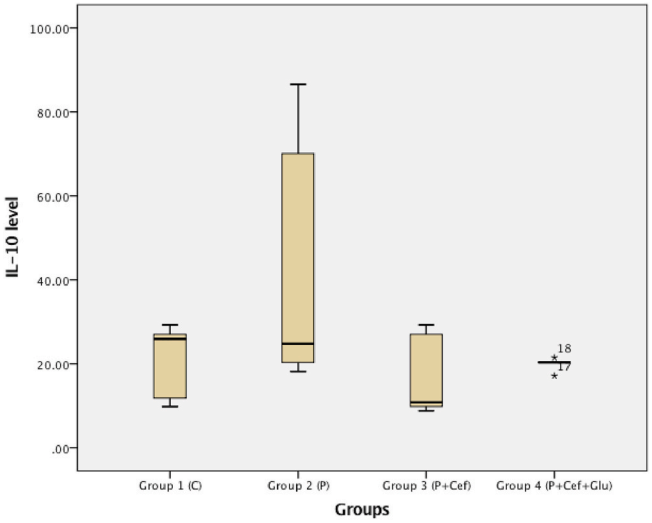

Fig. 2. Box-plot of IL-10 levels.

Table 3
Data distribution of IL-10/TNF-α ratio.

Parameter	Group	Min	Max	Mean ± SD	Median	p
IL-10/TNF-α ratio	1 (C)	0,08	2,43	0,97 ± 1,02	0,50	***0,20**
	2 (P)	0,01	0,48	0,16 ± 0,18	0,10	
	3 (P + Cef)	2,30	0,01	0,74 ± 0,89	0,38	
	4(P + Cef + Glu)	0,05	0,77	0,34 ± 0,25	0,32	

*one-way ANOVA test.

Glutathione supplementation was significantly decrease the mean level of TNF-α in rats induced peritonitis compared to the group 2 (peritonitis group) (p value 0,02) thus showing the anti-inflammatory effect of glutathione. It has been suggested that glutathione has anti-inflammatory effects by reducing the levels of pro-inflammatory cytokine productions. Glutathione blocked the activation of NF-κB pathway. This is the major pathway responsible for controlling inflammatory events through translocation of p50/p65 heterodimer to the nucleus. This event induces the production of pro-inflammatory cytokines such as TNF-α, IL-6 [19]. Other study also provided evidence that antioxidant provide anti-inflammatory function by block DNA-binding NF-κB and the expression of TNF-α and other pro-inflammatory cytokines, thereby blocking the cascade of inflammatory responses [20].

On the other hand, there was no significantly difference of mean TNF-α levels between group 3 (Peritonitis + Ceftriaxone) and group 4 (Peritonitis + Ceftriaxone + Glutathione) (p value 0,63). This showed that there was no significantly difference of TNF-α levels between peritonitis rats who received antibiotic only and received antibiotic + Glutathione as adjuvant therapy. In previous study established that antioxidant eliminates antibiotic-induced bacterial killing and also promote bacterial infection by decreasing the capacity of immune cells [21,22]. We suggest to do further investigation to determine the effective dose and timing of glutathione as adjuvant therapy in rats model peritonitis.

This study showed that there is no significantly difference in IL-10 levels in all rats (p value 0,75). However, there is similar pattern with TNF-α levels, the highest IL-10 value was also found in group 2 (peritonitis group). In Sewnath et al. study showed that peritonitis was associated with elevated IL-10 concentrations in both plasma and peritoneal fluid at 6 and 24 h after infection [23]. In previous human experiment which given *Escherichia coli* endotoxin intravenously, the mean TNF-α concentration was begin to increase at 1 h after endotoxin administration, peaked at 1,5 h. By contrast, the potent anti-inflammatory mediator IL-10 peaked at 3 h [24]. The increase of IL-10 concentration is the natural feedback factor to control inflammatory and immune responses [14]. However, in this study, we suggest that IL-10 failed to suppressed the release and activities of proinflammatory cytokines such as TNF-α.

During peritonitis, IL-10 can affect the host defense system in other ways than by inhibit TNF-α. It was reported that IL-10 can inhibit the expression of adhesion molecules in endothelial cells and reduce the function of proinflammatory neutrophils. Therefore, it is necessary to examine other mediators or cytokines to evaluate the IL-10 expression [23].

The mean levels of IL-10 was decrease in group 3 (Peritonitis + Ceftriaxone) and group 4 (Peritonitis + Ceftriaxone + Glutathione), whereas no significant difference between both groups. This result revealed that glutathione injection as adjuvant therapy has not yet effective in lower IL-10 levels in rats model peritonitis. Moreover, other study showed that antioxidant supplementation is related to decrease in phagocytosis and oxidative burst, that would disarm the host immune response and thus contribute to the increased bacterial load, leading to higher host mortality [7].

IL-10 is not only anti-inflammatory cytokine that inhibits the release of proinflammatory cytokines, but also limits injury due to an excessive inflammatory response mediated by proinflammatory cytokines. The fact that pro- and anti-inflammatory mediators are released simultaneously during the early phase of peritoneal sepsis noted in animal models of sepsis and septic patients [25,26]. However, a prolonged increase in IL-10 may suppress immune responses and aggravate the severity of disease. Therefore, IL-10/TNF-α ratio should be maintained within range 1,3–1,9 in order to have an anti-inflammatory effect [15]. Our result revealed that only one sample in group 3 within the proper range of IL-10/TNF-α ratio level (Table 3.) Overall ratio fell below the normal threshold, include group that received glutathione. This indicate that peritonitis rats that received glutathione had less anti-inflammatory

potential.

In bacterial infections, IL-10 acts as macrophage down-regulator/ macrophage inhibitors, reduces antigen presentation, prevents Th-1 cells from proliferating and suppresses production and TNF-α [27]. During peritonitis, these inflammatory cytokines can be determined not only in systemic circulation, but also has a considerable concentration in peritoneal exudate [14]. The release of inflammatory cytokines is mostly derived from macrophages that are exposed to bacterial endotoxins. Therefore, it is necessary to examined cytokine levels in peritoneal exudate.

The experimental model of bacterial peritonitis in rats was using *E. coli,* which the most common organism causes peritonitis and resembles a clinical condition with patients with bacterial peritonitis. The load of bacteria used was not lethal to rats. This model may represent useful tool to evaluate the efficacy of therapeutic intervention, as we evaluated the level of inflammatory cytokines to improve the prognosis patients with peritonitis.

We suggest to do further investigation to determine the effective dose and timing of glutathione as adjuvant therapy in rats model peritonitis, investigate whether combinations of peritoneal and circulating cytokine, or combinations with other markers as early prediction of disease severity.

5. Conclusion

In summary, glutathione supplementation is significantly decrease the mean level of TNF-α in rats induced peritonitis, however there is no difference compare to antibiotic only. Moreover, there is no significant changes level of IL-10 in rats induced peritonitis after glutathione injection. Further investigation with a longer time period of study is necessary to investigate and provide the effective dose of glutathione as adjuvant therapy in peritonitis.

Ethical approval

The experiment was approved by Research and Ethics Committee of Faculty of Medicine Diponegoro University, Indonesia (protocol number: 29/EC/H/FK-UNDIP/V/2020).

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Authors contributions

Study conception and design: Dila Junita, Endang Mahati, Agung Aji Prasetyo.

Data collection: Dila Junita.

Data analysis: Dila Junita, Endang Mahati.

Drafting the manuscript: Dila Junita, Agung Aji Prasetyo.

Critical revision of the manuscript: Dila Junita, Endang Mahati, Agung Aji Prasetyo, Muflihatul Muniroh, Tri Nur Kristina.

Provenance and peer review

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Declaration of competing interest

The authors have no financial conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.102406.

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