

Effect of glutathione administration as adjuvant therapy to NO and MDA level in Wistar rat peritonitis model



Ivani Yunita Korwa^{1*}, Mulflihatul Muniroh², Tri Nur Kristina³,
Agung Aji Prasetyo⁴, Endang Mahati⁵

¹Department of General Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia;

²Department of Physiology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia;

³Department of Clinical Microbiology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia;

⁴Department of Pediatric Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia;

⁵Department of Pharmacology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia;

*Corresponding author:

Ivani Yunita Korwa;
Department of General Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia;
ivaniyunitakorwa@gmail.com

Received: 2021-02-21

Accepted: 2021-07-20

Published: 2021-08-10

ABSTRACT

Background: Excessive amounts of free radicals beyond the antioxidant levels in the body will cause a condition called oxidative stress. Nitric oxide (NO) and malondialdehyde (MDA) are produced under conditions of oxidative stress. Glutathione (GSH) is the most endogenous antioxidant synthesized in humans. Therefore this study aims to prove whether the administration of glutathione as an adjuvant therapy can reduce levels of NO and MDA in peritonitis.

Methods: This is an experimental study with a post-test-only control group design. Twenty-four Wistar rats were randomly divided into four groups (6 rats/group). The first group (C) was a control group. The second group (P) was induced by peritonitis. The third group (P+Cef) was peritonitis-induced groups with given Ceftriaxone injection as therapy. The fourth group (P+Cef+Glu) was peritonitis-induced groups with given Ceftriaxone injection and glutathione as adjuvant therapy. After 24 hours, blood samples were taken for examination of NO and MDA levels. Data is considered significant if the p-value <0.05.

Results: There was a significant increase in NO level from Group 2 to Group 4 (P<0,05). There were no significant differences between MDA levels groups (P>0,05).

Conclusion: Glutathione as adjuvant therapy is not adequate to reduce NO and MDA levels in peritonitis.

Keywords: Glutathione, Peritonitis, NO, MDA.

Cite This Article: Korwa, I.Y., Muniroh, M., Kristina, T.N., Prasetyo, A.A., Mahati, E. 2021. Effect of glutathione administration as adjuvant therapy to NO and MDA level in Wistar rat peritonitis model. *Bali Medical Journal* 10(2): 713-716. DOI: 10.15562/bmj.v10i2.2227

INTRODUCTION

Peritonitis is a condition when there is microbial contamination in the peritoneal cavity.¹ Inflammation plays a crucial role in the defense response of the immune system against pathogen infection. Following the pathophysiology of peritonitis, when an inflammatory response is initiated by the binding of bacteria products (such as cell wall components to immune system receptors or receptors on endothelial cells), proinflammatory cytokines will release. This process results in the increasing production of reactive oxygen species (ROS).²

Oxidative stress is a general term for cellular damage caused by an imbalance between ROS and antioxidants levels, excessive amounts of ROS beyond the antioxidant levels in the body. Elevated ROS can produce cell dysfunction.^{2,3}

Excessive reactions from this process can cause systemic inflammatory response syndrome (SIRS), sepsis or sepsis shock.² Nitric oxide (NO) and MDA are produced when oxidative stress occurs.³⁻⁶ Patients with sepsis are exposed to severe oxidative stress, resulting from an oxidant-antioxidant imbalance.⁴ The oxidant and antioxidant state in septic patients has been evaluated in a recent study. They found that sepsis correlated with the severity of illness and patient outcome with the imbalance of oxidant and antioxidant levels.⁷

Glutathione (GSH) is the primary antioxidant supporting redox cells. It has been found in almost all parts of the cell: cytosol, endoplasmic reticulum and mitochondria.² Tripeptide GSH (-glutamyl-cysteinyl glycine) is a compound that is abundant in tissues.⁸ Glutathione performs various functions. The primary protective roles of GSH

against oxidative stress are: (i) it is a cofactor of several detoxifying enzymes against oxidative stress, e.g., Glutathione peroxidase and GSH transferase, (ii) it participates in amino acid transport through the plasma membrane, and it scavenges ·OH and singlet oxygen directly, detoxifying H₂O₂ and lipid peroxides by the catalytic action of GPx.²

The administration of glutathione as an antioxidant supplement is expected to be an additional availability of antioxidants inside the body in dealing with oxidative stress in patients with peritonitis. It is hoped that it will prevent the patient from falling into a state of sepsis by preventing oxidative stress. This study determined the NO and MDA level in peritonitis with the administration of glutathione as an adjuvant therapy.

METHOD

Experimental Animals

Twenty-four male Wistar rats weighing between 200 and 300 g were used in this experiment (inbred at Animal house of Setia Budi University, Solo, Indonesia). The Wistar rats were kept under standard laboratory conditions at the Animal Laboratory of Setia Budi University, Solo, Indonesia. The samples were kept at room temperature between 26 – 30°C with a 12 h light/dark cycle. All animals were fed with commercial rat feed and water ad libitum. Animal Ethics Committee of Faculty of Medicine, Diponegoro University, Semarang, Indonesia, approved this study (protocol number: 30/EC/H/FK-UNDIP/V/2020).

Peritonitis Induction

Peritonitis was induced with *Escherichia coli* suspension 10^7 CFU/ml as much as 1.5 ml intraperitoneally.

Animal Group and Study Design

The animals were divided into four groups; each group consists of 6 rats. The first group (C) was the control group that was not induced by peritonitis. The second group (P) was induced by peritonitis. The third group (P + Cef) was the peritonitis-induced group with Ceftriaxone (186 mg/kg of body weight) administration (intravenously) at 1 hour after peritonitis induction. The last group (P + Cef + Glu) was the peritonitis-induced group with Ceftriaxone and Glutathione (250 mg/kg of body weight) administration (intravenously) at 1 hour after induction of peritonitis. The doses of Ceftriaxone and Glutathione were calculated and converted from human to rat doses. The route and method of administration have also been adjusted to the guidelines for maximum injection volume by species, site location and gauge size.⁹ Twenty-four hours after all treatments were carried out, and all blood samples were collected to determine NO and MDA levels. After blood sample collecting, the rats were killed.

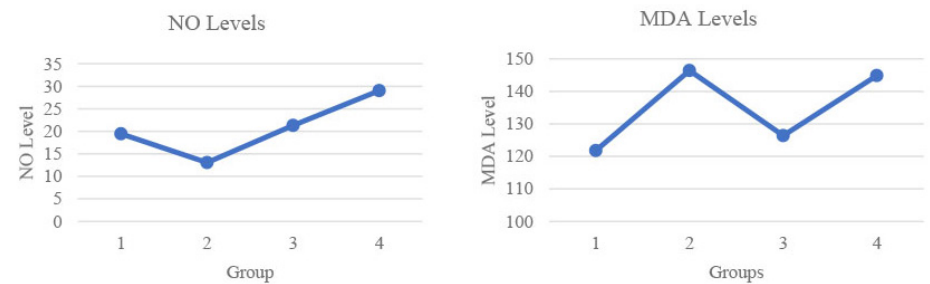
Nitric oxide and MDA Level Determination

Nitric oxide levels were determined using the NO Assay kit that was manufactured by Elabscience®. Malondialdehyde levels

Table 1. Data Distribution of the NO and MDA Levels in Experimental Group

| Group | NO Level | | MDA Level | |
|--------------|-------------------|-----------------|---------------------|-----------------|
| | Mean±SD (µg/L) | p value (ANOVA) | Mean±SD (µmol/L) | p value (ANOVA) |
| C | 19,4425 ± 3,79270 | 0,013* | 121,7775 ± 14,75361 | 0,342 |
| P | 13,0750 ± 5,38973 | | 146,4100 ± 23,05485 | |
| P + Cef | 21,3000 ± 6,43532 | | 126,3800 ± 31,08644 | |
| P+ Cef + Glu | 29,0500 ± 6,37626 | | 144,8750 ± 18,80916 | |

NO = Nitric oxide, MDA = Malondyaldehyde, C = Control, P = Peritonitis, P + Cef = Peritonitis + Ceftriaxone, P + Cef + Glu = Peritonitis + Ceftriaxone + Glutathione. * = p value < 0,05.



NO = Nitric oxide, MDA = Malondyaldehyde, Group 1 = C (Control), Group 2 = Peritonitis (P), Group 3 = Peritonitis + Ceftriaxone (P + Cef), Group 4 = Peritonitis + Ceftriaxone + Glutathione (P + Cef + Glu)

Figure 1. Line Diagram of NO and MDA Levels.

were determined using an ELISA kit that was manufactured by Elabscience®. The analysis was following the instructions that were provided by the manufacture. The analysis was executed in GAKI Laboratorium, Diponegoro University, Semarang, Indonesia. The results were expressed as µg/L and µmol/L respectively for NO and MDA.

Statistical Analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS), Version 26.0 for Mac Software. Nitric oxide levels data were compared using the t-test for impaired data with an analysis of variance ANOVA. Malondialdehyde levels data were compared using the t-test for impaired data with an analysis of variance Kruskal-Wallis test. Data were considered significant if the p-value was ≤ 0.05.

RESULT

All Wistar rats survived in this study. The NO and MDA levels in the experimental

group are shown in Table 1. The highest average of NO levels was achieved in Group 4 (P + Cef + Glu), while Group 2 (P) was the lowest. Group 2 (P + Cef + Glu) achieved the highest MDA levels, while the lowest was Group 1 (C).

There were significant differences between NO levels groups (p=0,013, p<0.05). There were no significant differences between MDA levels groups (p=0,342, p>0.05).

We found that there was an enhancement of MDA level from Group 1 (C) to Group 2 (P), but there was decreasing in MDA level from Group 2 (P) to Group 3 (P + Cef). In contrast, there was decreasing in NO level from Group 1 (C) to Group 2 (P), but there was an enhancement of NO level from Group 2 (P) to Group 3 (P + Cef) that as shown in Figure 1.

Based on Bonferroni's multiple comparison tests, there is a significant increase of NO levels between Group 2 (P) and Group 4 (P + Cef + Glu) (Table 2).

Table 2. Bonferroni Multiple Comparison Test for NO Levels.

| Group | C | P | P + Cef | P + Cef + Glu |
|---------------|---|-------|---------|---------------|
| C | - | 0,803 | 1,000 | 0,192 |
| P | - | - | 0,306 | 0,016* |
| P + Cef | - | - | - | 0,444 |
| P + Cef + Glu | - | - | - | - |

C = Control, P = Peritonitis, P + Cef = Peritonitis + Ceftriaxone, P + Cef + Glu = Peritonitis + Ceftriaxone + Glutathione. * = p value < 0,05.

DISCUSSION

Following the theoretical basis discussed earlier, NO and MDA are both produced when oxidative stress occurs. However, in this study, we found an increase of MDA levels accompanied by decreasing NO levels in Group 2 (P). These results are similar to El-Ashker et al., who compared MDA levels in horses with peritonitis (survivors and non-survivors). They found a significant increase in MDA levels ($p < 0.05$) and a significant decrease in NO levels in the non-survivor group compared to the survivor group.¹⁰ These results were also similar to Mishra et al. Regarding NO and MDA levels in DM patients type 2. Oxidative stress has become an actual entity in the etiopathogenesis of type 2 diabetes mellitus (DM). They found an increase in MDA levels and decreased NO levels in patients with DM compared to the control group (non-DM). Malondialdehyde (MDA) levels show a significant positive correlation with plasma glucose. NO levels correlate significantly with plasma glucose levels.¹¹ During the inflammatory process, there will be the release of inflammatory mediators between TNF- α . Tumor necrosis factor- α (TNF- α) is mainly produced in adipocytes and/or peripheral tissue. Increased TNF- α levels induce insulin resistance in adipocytes and peripheral tissues by interfering with insulin signaling through serine phosphorylation, leading to insulin resistance development.¹² Serum phosphorylation of insulin substrate-1 receptor (IRS-1) induced by TNF- α causes inhibition of receptor receptors. Insulin and reduces the activity of the phosphatidylinositol-3 kinase, which resulted in insulin resistance.¹³ Tumor necrosis factor-alpha induces decreased expression of glucose transporter 4 (GLUT 4).¹³ In addition, NO production

is stimulated by insulin.¹² Bioavailability of NO is strongly influenced by oxidative stress induced by hyperglycemia. Insulin leads to high expression and NO synthesis activity.¹⁴ This condition may explain the disturbance of NO levels in Wistar model peritonitis mice in our study. Research conducted by Winkler et al. found similar results. They observed an association between disease severity and systemic inflammation and sepsis and found that NO's bioavailability was reduced.⁵ However, if Group 2 (P) was compared with Group 1 (C), the decreasing of MDA levels and the increase of NO levels were not significant (p-value >0.05) (Table 1 and 2).

This study also found that administering antibiotics (Ceftriaxone) to the Wistar rat model of peritonitis could reduce MDA levels, accompanied by the increase of NO levels. It can be concluded that there are decreasing of inflammatory process caused by the bactericidal effect of antibiotics. The results of this study are in line with research conducted by Yildirim et al., who also found that MDA levels decreased in the antibiotic-treated peritonitis group.¹⁵ However, if Group 3 (P+Cef) compared with Group 2 (P), the decreasing of MDA levels and the increase of NO levels were not significant (p-value >0.05).

The previous finding suggests that antioxidants' administration can significantly reduce oxidative stress (NO and MDA levels) in peritonitis.¹⁵⁻¹⁸ In this study, it was found that there was an increase of NO and MDA levels in Group 4 (P+Cef+Glu). It showed the ineffectiveness of glutathione as adjuvant therapy. This result is related to research conducted by Goswani, et al. They found that the administration of antioxidants in rats induced by peritonitis

can significantly increase bacteria number. The antioxidant could inhibit the process of bacterial phagocytosis.¹⁹ They also found that administration of glutathione could reduce TNF- levels (significantly) by 12-25% in mice treated with peritonitis.¹⁹ The decrease in TNF levels was related to an increase in NO levels (as explained earlier).^{12,13} This is also in line with the review that Rocha presented, et al. They identified that the target cell damage caused by free radicals was the presence of mitochondrial dysfunction.² Allen et al. conducted a clinical trial by giving oral glutathione to healthy adults, then measuring the level of oxidative stress (represented by levels of F2-isoprostanes (F2-isoP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) urine) and levels of GSH, GSSG, and GSH-GSSG ratio. They also found no significant changes in oxidative stress levels or body glutathione status.²⁰ Therefore, the increasing of MDA levels can be caused by the antioxidants distributed throughout the body but not accumulate in the mitochondria the administration of antioxidants becomes ineffective. However, if Group 4 (P+Cef+Glu) compared with Group 3 (P+Cef), the increase of MDA and NO levels was not significant (p-value >0.05).

There was some limitation in our study. First, we did not measure MDA and NO levels in groups of experimental animals before treatment. Second, we did not measure GSH levels circulating in the circulation before and after manipulation, so we did not see the effectiveness of giving glutathione. Third, we only used glutathione in a single dose in this study, so we could not determine an effective Glutathione dose in reducing oxidative stress. Moreover, we did not measure blood glucose levels in the treatment group, so we could not directly see the effect of hyperglycemia on changes in MDA and NO levels.

CONCLUSION

Glutathione administration as adjuvant therapy is ineffective in reducing NO and MDA levels in Wistar peritonitis model rats.

DISCLOSURE

Conflict of Interest

All authors declared that there is no conflict of interest.

Funding Sources

The source of funding in this research comes from personal funds.

Author Contribution

All authors in this manuscript contributed equally to conducting the research and preparing for publication.

REFERENCE

1. Beilman GJ DD. Surgical Infection. In: Anderson DK BT, Dunn DL, Hunter JG, Matthews JB, Pollock RE, editor. Schwartz's principle of surgery. 10th ed. New York: Mc Graw Hill; 2015. p. 149.
2. Rocha M HR, Rovira S, Hernandez-Mijares A, Victor VM. . Mitochondrial dysfunction and antioxidant therapy in sepsis. *Infectious Disorders- Drug Targets*. 2012;12:161-78.
3. Mantzaris K TV, Zakyntinos E. Role of oxidative stress and mitochondrial dysfunction in sepsis and potential therapies. *Oxid Med Cell Longev*. 2017;10.
4. Daga MK KN, Singh H, Chhoda A, Mattoo S, Gupta BK. Markers of oxidative stress and clinical outcome in critically ill septic patients: a preliminary study from North India. *J Clin Diagn Res*. 2016;10(8):35-8.
5. Winkler M, Kluge S, Holzmann M, Moritz E, Robbe L, Bauer A, et al. Markers of nitric oxide are associated with sepsis severity: an observational study. *Critical Care*. 2017;21(1):1-9.
6. Lorente L, Martin MM, Abreu-Gonzalez P, Dominguez-Rodriguez A, Labarta L, Diaz C, et al. Sustained high serum malondialdehyde levels are associated with severity and mortality in septic patients. *Crit Care*. 2013;17(6):R290.
7. Guerreiro MO, Petronillo F, Andrades M, Constantino L, Mina F, Moreira J, et al. Plasma superoxide dismutase activity and mortality in patients with septic. *Journal of Trauma - Injury, Infection, and Critical Care*. 2010;69:E102-6.
8. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions. *J Amino Acids*. 2012;2012:736837.
9. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm*. 2016;7(2):27-31.
10. El-Ashker M, El-Khodery S, El-Boshy M, Mohamed A. Prognostic significance of lipid peroxide and antioxidant levels in draft horses with peritonitis. *Comp Clin Pathol* 2011;20:433-9.
11. Mishra S, Mishra B. Study of Lipid Peroxidation, Nitric Oxide End Product, and Trace Element Status in Type 2 Diabetes Mellitus with and without Complications. *Int J Appl Basic Med Res*. 2017;7:88-93.
12. Akash M, Rehman K, Liaqat A. Tumor Necrosis Factor-Alpha: Role in Development of Insulin Resistance and Pathogenesis of Type 2 Diabetes Mellitus. *Journal of Cellular Biochemistry*. 2017;199(1):105-10.
13. Lakić I, Belić B, Cincović M, et al. Relationship of Circulating Tumor Necrosis Factor Alpha (TNF- α) and Insulin Secretion and Resistance in Euglycaemic Dogs. *Acta Scientiae Veterinariae*. 2020;48:1731.
14. Tessari P, Cecchet D, Cosma A, et al. Nitric oxide synthesis is reduced in subjects with type 2 diabetes and nephropathy. *Diabetes*. 2010;59:2152-9.
15. Yildirim Y, Cellad EG, Kara AV, Yilmaz Z, Kadiroglu AK, Bahadir MV, et al. Effect of intraperitoneal etanercept on oxidative stress in rats with peritonitis. *Oxid Med Cell Longev*. 2016;2016:9418468.
16. Altincik A SF, Yenisey C, Duman S, Can A, Akev N, Kirdar S, et al. Effects of Aloe vera leaf gel extract on rat peritonitis model. *Indian Journal of Pharmacology*. 2014;46(3):322-7.
17. Falcao TR AA, Soares LAL, Ramos, TM, Bezerra ICF, Ferreira MRA, et al. Crude extract and fractions from *Eugenia uniflora* Linn leaves showed anti-inflammatory, antioxidant, and antibacterial activities. *BMC Complementary and Alternative Medicine*. 2018;84:1-12.
18. Falcão T, de Araújo A, Soares L, de Farias I, da Silva W, Ferreira M, et al. Libidibia ferrea fruit crude extract and fractions show anti-inflammatory, antioxidant, and antinociceptive effect in vivo and increase cell viability in vitro. *Evidence-Based Complementary and Alternative Medicine*. 2019:1-13.
19. Goswami M, Sharma D, Khan NM, Checker R, Sandur SK, Jawali N. Antioxidant supplementation enhances bacterial peritonitis in mice by inhibiting phagocytosis. *J Med Microbiol*. 2014;63(Pt 3):355-66.
20. Allen J, Bradley RD. Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. *J Altern Complement Med*. 2011;17(9):827-33.



This work is licensed under a Creative Commons Attribution