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Protective effect of a combined glutathione and prednisolone administration on mice subjected to a lipopolysaccharide-induced cytokine storm

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ABSTRACT

Cytokine storm is a life-threatening hyperinflammatory condition that can result from infectious diseases, including COVID-19, as well as from non-infectious autoimmune disorders. This study has investigated the potential of glutathione to mitigate a cytokine storm in mice, particularly when administered in combination with prednisolone; a synthetic glucocorticoid. Fifty male albino mice were randomly assigned into five groups. The negative control group received 1% dimethyl sulfoxide (DMSO) intraperitoneally (i.p.), while the positive control group received a single i.p. dose of lipopolysaccharide (LPS) at 5 mg/kg. The treatment groups received either glutathione (200 mg/kg i.p.), prednisolone (5 mg/kg i.p.), or a combination of both drugs, each administered as a single dose, 1 h prior to the LPS injection. After 24 h, blood samples were collected in order to assess the serum interleukin-6 (IL-6) levels, and lung tissues were harvested for histopathological analysis. The results demonstrated that glutathione, prednisolone, and their

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combination can significantly reduce the LPS-induced elevation of IL-6 levels (p<0.05) and can ameliorate the LPS-induced histopathological damage in the lung tissues of mice. In conclusion, pre-treatment with glutathione, prednisolone, or their combination can effectively attenuate both the systemic IL-6 elevation and the pulmonary histopathological alterations associated with cytokine storm. Notably, the combined administration of glutathione and prednisolone exhibited synergistic protective effects, suggesting their potential utility as prophylactic agents against cytokine storm syndromes.

1. Introduction

The term "cytokine storm" encompasses a group of immune-mediated dysregulated conditions characterized by systemic inflammation and widespread tissue damage, which, if left unmanaged, may culminate in multi-organ failure[1]. Among the pro-inflammatory mediators, interleukin-6 (IL-6) plays a central role; its serum concentration remains elevated during sustained inflammatory responses, whereas levels of other cytokines typically decline more rapidly[2].

Lipopolysaccharides (LPS), large molecules composed of lipid and polysaccharide components, are found in the outer membrane of Gram-negative bacteria and are known to trigger potent inflammatory responses[3]. Exposure to LPS has been implicated in the pathogenesis of various inflammatory diseases.

Glutathione is a naturally occurring tripeptide with flavanol-like properties, deriving from plant sources. It exhibits potent antioxidant, anti-inflammatory, and immunomodulatory activities, with a favourable safety profile[4]. In contrast, corticosteroids – long used for their immunosuppressive and anti-inflammatory effects – are associated with significant adverse effects, particularly at high doses or with prolonged use[5]. Given these considerations, the present study aimed at evaluating the anti-cytokine storm potential of glutathione, both alone and in combination with prednisolone; a synthetic corticosteroid.

2. Methodology

2.1. Drugs and chemicals

Glutathione and prednisolone (dry powder form) were obtained from Hangzhou Hyper Chem Ltd (China). LPS was purchased from Sigma-Aldrich (Germany). All biochemical, haematological, and IL-6 assay kits were sourced from Roche (Germany) and CUSABIO (USA).

2.2. Preparation of treatment solutions

Fresh stock solutions of glutathione were prepared at concentrations of 200 and 50 mg/kg by dissolving the compound in 1% dimethyl sulfoxide (DMSO), followed by dilution with distilled water. Prednisolone stock solutions (5 and 1.25 mg/kg) were similarly prepared using 1% DMSO.

2.3. Induction of cytokine storm

Cytokine storm was induced by an intraperitoneal (i.p.) injection of (*Escherichia coli*-derived) LPS at 5 mg/kg, administered in a 300-µL volume. The LPS stock solution was prepared at a concentration of 1 mg/mL, following the manufacturer's instructions[6].

2.4. Animal groups and experimental design

Fifty male albino Swiss mice (25–35 g) were randomly divided into five groups (n=10 per group): (i) negative control group (received 1% DMSO i.p.), (ii) positive control (LPS) group (received LPS at 5 mg/kg i.p.), (iii) glutathione group (received glutathione at 200 mg/kg i.p., 1 h before LPS), (iv) prednisolone group (received prednisolone at 5 mg/kg i.p., 1 h be-

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Table 1. Injury scores conferred by the histopathological assessment of murine tissues stained with haematoxylin and eosin. Abbreviations used: LPS, lipopolysaccharide; SD, standard deviation.

Groups (n=10 mice each)	**Injury score in tissues** (mean ± SD)				***p*-value**
	liver	**kidney**	**heart**	**lung**	
negative control	0	0	0	0	--
positive control (LPS)	8 ± 2.7	4 ± 1.5	2 ± 1.3	5 ± 1.4	<0.001 *vs.* negative control group
glutathione + LPS	1 ± 1.5	1 ± 1.3	1 ± 1.4	2 ± 1.3	<0.05 *vs.* positive control (LPS) group
prednisolone + LPS	3 ± 1.2	1 ± 0.5	1 ± 1.9	2 ± 1.6	<0.01 *vs.* positive control (LPS) group
glutathione + prednisolone + LPS	1 ± 0.7	1 ± 0.4	1 ± 1.2	1 ± 0.7	<0.001 *vs.* positive control (LPS) group

fore LPS), and (v) drug combination group (received a 25% of the aforementioned doses of glutathione and of prednisolone, i.p. 1 h before LPS).

2.5. IL-6 levels' measurement

At 24 h post-induction, blood samples (1–1.5 mL) were collected from the jugular vein under chloroform anaesthesia into Eppendorf tubes, and were allowed to clot for 15 min. Samples were centrifuged at 3,000 rpm for 10 min, and serum IL-6 levels were quantified using an enzyme-linked immunosorbent assay (ELISA). Following blood collection, all animals were euthanized *via* cervical dislocation for the collection of lung tissue samples and the undertaking of histopathological analysis.

2.6. Histopathological evaluation

Tissues were fixed, embedded in paraffin, and sectioned at an average thickness of 5 μm using a rotary microtome. Sections were deparaffinized using heat, xylene, and graded ethanol, were mounted on clean slides, and were stained with haematoxylin and eosin for microscopic examination.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using the SPSS software (version 20). A *p*-value below 0.05 was considered statistically significant.

2.8. Ethics approval

This study was approved by the Institutional Review Board of the College of Pharmacy of Al-Nahrain University, under document IRB/204 and approval code UNCOPIRB202402242 (dated: 24 February 2024). The protocol was reviewed in accordance with the latest ethical guidelines and research standards.

3. Results and Discussion

Serum IL-6 levels were quantified using ELISA and expressed in pg/mL. The positive control group (LPS-treated) exhibited a statistically significant elevation in IL-6 levels compared to the negative control group ($p<0.001$). Pre-treatment with glutathione, prednisolone, and their combination resulted in a significant ($p<0.05$), highly significant ($p<0.01$), and very highly significant ($p<0.001$) reduction in IL-6 levels, respectively, when compared to those of the LPS-only-treated group.

LPS is known to activate the expression of multiple pro-inflammatory cytokines and to increase the production of reactive oxygen species (ROS), thereby contributing to the development of a cytokine storm[6]. Previous studies have shown that both glutathione and prednisolone can attenuate IL-6 levels in LPS-induced cytokine storm models[7]. Notably, in the present study, a combination of reduced doses (25%) of glutathione and prednisolone was as effective as the full-dose monotherapies in significantly lowering the serum IL-6 levels in mice exposed to LPS, thereby suggesting a synergistic interaction.

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The histopathological examination of lung tissue sections stained with haematoxylin and eosin has further supported these findings. The negative control group displayed normal pulmonary architecture, with intact alveoli and alveolar spaces (injury score = 0). In contrast, the LPS-treated group exhibited marked pathological changes, including vascular congestion, dense inflammatory cell infiltration, and destruction of the alveolar septa (mean injury score = 5). The glutathione-treated group showed congestion with haemorrhage and mild thickening of interalveolar septa, along with modest inflammatory cell infiltration (mean injury score = 2). On the other hand, the prednisolone-treated group exhibited slight congestion and localized septal thinning, with mild inflammatory cell infiltration (mean injury score = 2). Finally, the group treated with the drug combination demonstrated minimal inflammatory cell infiltration and slight congestion in the interalveolar septa (mean injury score = 1), thereby indicating the most pronounced histological improvement (Table 1).

The histopathological changes observed in the LPS group were very highly significant compared to those of the control group. The observed tissue damage is consistent with oxidative stress-induced inflammation, which can perpetuate a cycle of cellular injury and immune activation[8]. LPS is known to interact with toll-like receptors, activate the nuclear factor kappa B (NF-κB) pathway, and promote the release of pro-inflammatory cytokines, nitric oxide, and ROS, leading to macrophage activation and inflammatory cell recruitment[9]. The lungs, due to their extensive surface area and vascular exposure, are particularly susceptible to inflammatory and oxidative insults. Additionally, the pulmonary capillary bed may receive cytokine-rich blood from the liver following hepatic injury, potentially explaining the lung's vulnerability to secondary damage. This aligns with previous studies reporting significant histopathological alterations in the lungs following LPS administration in murine models[10].

Importantly, the pre-treatment groups (i.e., those receiving glutathione, prednisolone, or their combination) have exhibited statistically significant improvements (p<0.05) in their lung histology compared to that of the LPS-only-treated group. The protective mechanism likely involves glutathione's ability to downregulate NF-κB expression and suppress oxidative stress, thereby reducing IL-6 production[7,10]. Overall, the combined assessment of IL-6 levels and histopathological findings confirms that a pre-treatment with glutathione, prednisolone, or their combination through a single i.p. dose can confer substantial protection against the LPS-induced cytokine storm in mice.

4. Conclusion

A pre-treatment with glutathione and prednisolone can effectively mitigate the LPS-induced cytokine storm in mice, by reducing serum IL-6 levels and preserving lung tissue integrity. Notably, the combination of glutathione and prednisolone exhibits a synergistic protective effect, suggesting its potential as a corticosteroid-sparing strategy for managing cytokine storm-related pathologies.

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Conflicts of interest

None exist.

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References

1. Omran Z.S, Sahib H.B, Radhi A.J, Ali N.M. *In silico* design, and *in vivo* evaluation of synthesized phenyl isoserine derivative and used as anticytokine storm. *Egypt. J. Chem.* 66(5), 151–159, 2023. DOI: 10.21608/ejchem.2022.144346.6319
2. Shaker N.S., Sahib H.B., Tahseen N.J. Anti-cytokine storm activity of fraxin, quercetin, and their combination on lipopolysaccharide-induced cytokine storm in mice: implications in COVID-19. *Iran. J. Med. Sci.* 49(5), 322–331, 2024. DOI: 10.30476/ijms.2023.98947.3102
3. Aal-Aaboda M.S., Abu Raghif A.R., Hadi N.R. Renoprotective potential of the ultra-pure lipopolysaccharide from Rhodobacter sphaeroides on acutely injured kidneys in an animal model. *Arch. Razi Inst.* 76(6), 1755–1764, 2021. DOI: 10.22092/ari.2021.356202.1803
4. Hsieh I.T., Liao C.C., Chen J.H., Yang C.C., Chou T.H., Nagarajan D., *et al.* Enhanced stability, antioxidant capacity and *in vivo* anti-inflammatory efficacy of glutathione and quercetin *via* nanoemulsion formulation. *J. Taiwan Inst. Chem. Eng.* 168, 105943, 2025. DOI: 10.1016/j.jtice.2024.105943
5. Alhashem G., Raghif A.R.A., Abbas A.A. The effect of liraglutide on biochemical markers of bone turnover in rats treated by dexamethasone. *Int. J. Drug Deliv. Technol.* 12(2), 598–602, 2022.
6. Al-Dabbagh M.A, Sahib H.B. Naringenin ameliorates lipopolysaccharide-induced kidney and lung injuries in mice: Decisive role of IL-1β, IL-6, IL8, and TNF-α. *Opera Med. Physiol.* 11(1), 34-51, 2024. DOI: 10.24412/2500-2295-2024-1-34-51
7. Aggarwal S., Dimitropoulou C., Lu Q., Black S.M., Sharma S. Glutathione supplementation attenuates lipopolysaccharide-induced mitochondrial dysfunction and apoptosis in a mouse model of acute lung injury. *Front. Physiol.* 3, 161, 2012. DOI: 10.3389/fphys.2012.00161
8. Yahiya Y.I., Hadi N.R., Abu Raghif A., Qassam H., Al Habooby N.G.S. Role of Iberin as an anti-apoptotic agent on renal ischemia-reperfusion injury in rats. *J. Med. Life* 16(6), 915–919, 2023. DOI: 10.25122/jml-2022-0281
9. Raheem A.K., Abu-Raghif A.R., Zigam Q.A. Cilostazol protects against sepsis-induced kidney impairment in a mice model. *J. Med. Chem. Sci.* 6(5), 1193–1203, 2023. DOI: 10.26655/JMCHEMSCI.2023.5.25
10. Abed-Mansoor A.F., Abu-Raghif A.R. Attenuated effects of rivastigmine in induced cytokine storm in mice. *J. Emerg. Med. Trauma Acute Care* 2022(3), 12, 2022. DOI: 10.5339/jemtac.2022.ismc.12

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