



Effect of Diclofenac on Hematological Parameters and Inflammatory Markers in Rat after Injection of *Escherichia coli* Lipopolysaccharide

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Abstract

Background: Bacterial lipopolysaccharide (LPS) is a large pathogen-associated molecule that affects both animals and humans.

Objective: The aim of this study was to assess the effect of diclofenac sodium on hematological parameters and inflammatory markers after intraperitoneal injection of LPS in rats.

Materials and Methods: Ninety-six male Wistar rats were divided randomly into 8 equal groups. Groups I, II, and III were only injected intraperitoneally (IP) with 100, 200, and 300 µg/kg LPS, respectively. Groups IV, V, and VI were injected with LPS at doses similar to the above groups plus diclofenac 2.5 mg/kg (IM). Group VII was injected only with diclofenac at the same dose and group VIII (control group) was injected with normal saline. Blood samples were collected from the rats in different times (0, 1, 6, and 24 hours) after injection.

Results: The results showed that white blood cell (WBC), neutrophil, and lymphocyte counts significantly decreased in all groups at 1 and 6 hours after injection of LPS ($P < 0.05$). The total leukocyte count, neutrophils, and lymphocytes increased at 24 hours after injection of LPS, in groups I, II, III, and VI ($P < 0.05$). The C-reactive protein (CRP) levels at 6 and 24 hours after injection of LPS, in groups I, II, III, and VI, showed significant changes ($P < 0.05$). The CRP level decreased in groups IV, V and VI (LPS + diclofenac) compared with the groups that were not injected with diclofenac ($P < 0.05$). A significant increase was seen in fibrinogen level in all challenged groups (with or without diclofenac) at 24 hours after injection of LPS ($P < 0.05$).

Conclusion: Injection of diclofenac together with LPS did not affect the leukocyte changes (total and different count) and plasma fibrinogen level in rats. Diclofenac was effective in preventing high CRP changes induced by injection of LPS.

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Background

Lipopolysaccharides (LPSs) are found in the outer membranes of gram-negative bacteria, such as *Salmonella* and *Escherichia coli*.¹ Injection of LPS evokes proinflammatory response, and in the blood may result in fever, shock, organ failure, and death.² LPS is used in the modeling of endotoxemia in experimental models.³ Two cyclooxygenase enzymes are known. Cyclooxygenase-2 (COX-2) regulates release of prostaglandins important for the inflammatory response.⁴ Expression of COX-2 is increased after exposure to LPS.⁵ Monitoring the serum levels of cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), or tumor necrosis factor- α (TNF- α) may be an effective method for the evaluation of inflammatory response and early detection of acute phase reactions.⁶⁻⁸ Pro-inflammatory cytokines, such as TNF- α and IL-6 were induced by endotoxins like LPS, leading to inflammation and coagulation cascades. These cytokines may trigger the

expression of tissue factors in monocytes and endothelial cells.⁹ C-reactive protein (CRP) is produced by the liver in response to inflammation regulated by IL-6, IL-1, and TNF- α .¹⁰ IL-6 in LPS challenge and inflammation induces weight loss, hypoglycemia and fibrinogen production.¹¹

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used as analgesics, anti-inflammatory drugs, and antipyretics.¹² NSAIDs block the action of COX, thereby reducing symptoms of inflammation.¹³ Ruetten and Thiemermann in a recent study demonstrated that the expression of COX-2 caused by LPS in the rat was prevented by dexamethasone.¹⁴ Yazar et al showed that flunixin meglumine inhibited the increase of inflammatory cytokine levels (TNF α and IL-1) in LPS-induced endotoxic mice.¹⁵

Diclofenac, as an NSAID, has been widely used as the anti-inflammatory, analgesic, and antipyretic drug. The potency of diclofenac is substantially greater than that

of indomethacin, naproxen, and several other agents.¹⁶⁻¹⁹ *E. coli* LPS is one of the most potent stimuli for cytokine release and is used as an experimental model.

Objective

The objective of the present work was to study the effect of diclofenac on hematological parameters and inflammatory markers during intraperitoneal injection of *E. coli* LPS in rats.

Materials and Methods

Animal and Experimental Design

Ninety-six male Wistar rats were used in this study. The rats were divided randomly into 8 equal groups. Groups I, II, and III were injected intraperitoneally with 100, 200 and 300 µg/kg LPS from *E. coli* (Serotype 0111:B4; Sigma-Aldrich, USA). Groups IV, V, and VI were injected with LPS at a dose similar to the above groups, plus diclofenac (75 mg, Caspian Co., Iran) at a dose of 2.5 mg/kg, intramuscularly (IM). Group VII received only diclofenac at the same dose. Group VIII (control group) received normal saline. Blood samples were collected from the heart at 0, 1, 6 and 24 hours and collected into 3.8% sodium citrate tubes (Sigma-Aldrich, USA). Blood samples were collected to assay the hematological and inflammatory parameters. The sera were separated and stored at -20°C.

Analysis of Hematological Parameters and Inflammatory Markers

Blood smears were prepared for total and differential leukocyte count. White blood cells were counted by Neubauer haemocytometer. Differential leukocyte count was performed with blood smears stained with Giemsa (Labtron Co., UK). CRP and fibrinogen levels were measured by kits (Mahsayaran Co., Iran), (Parsazmoon Co., Iran) using Clauss assay and an Immunoturbidimetry assay, respectively.

Table 1. Effect of Diclofenac on Total Leukocyte Count (µL) in Rat After Intraperitoneal Injection of Lipopolysaccharide

Groups	Time (h)			
	0	1	6	24
I	7967±288	4430±210	3867±165	7667±165
II	7733±251	4766±177	4233±134	7133±166
III	7957±289	4167±209	3816±128	8077±172
IV	7867±255	4443±221	3800±159	7350±188
V	7869±159	4367±171	3750±174	7600±196
VI	8067±243	4380±186	3850±142	6950±233
VII	7833±181	7800±262	7767±225	7700±244
VIII	7932±192	7780±210	7866±216	7716±198

Significance level, $P < 0.05$.

Statistical Analysis

Statistical analysis was performed using SPSS version 16.0. Duncan's post hoc test was used for comparison of groups and analyses of variance. $P < 0.05$ was considered statistically significant.

Results

The results showed that LPS reduced WBC count at 1 and 6 hours after injection of LPS compared with control group, mainly due to a decrease in neutrophil and lymphocyte counts (Tables 1 and 2) ($P < 0.05$). In LPS group, the total leukocyte count, neutrophils and lymphocytes increased at 24 hours ($P < 0.05$) (Tables 1 and 2). Pretreatment with diclofenac did not change hematological parameters. CRP was significantly increased in LPS group after 6 and 24 hours ($P < 0.05$). The levels of CRP in diclofenac-treated groups were decreased ($P < 0.05$) (Table 3). Fibrinogen was relatively increased in LPS group. It was not decreased in the treated groups Compared with LPS group ($P < 0.05$) (Table 4).

Discussion

LPS is the main component of the outer membrane of

Table 2. Effect of Diclofenac on Neutrophil (N) and Lymphocyte (L) Counts (µL) in Rat After Intraperitoneal Injection of Lipopolysaccharide

Groups	Time			
	0 N /L	1 h N /L	6 h N /L	24 h N /L
I	1925±79, 5482±110	790±42, 3615±124	370±16, 3142±136	1910±65, 5253±125
II	1981±87, 5126±116	861±39, 3741±120	458±19, 3485±128	1790±62, 5016±117
III	2020±79, 5336±127	710±40, 3511±143	338±18, 3256±122	2010±66, 5433±125
IV	1898±81, 5425±136	802±36, 3547±142	343±15, 3266±139	1820±59, 5275±109
V	1992±77, 5300±145	785±41, 3390±128	378±12, 3050±136	1890±57, 5228±133
VI	2010±79, 5512±160	772±36, 3532±115	372±18, 3212±142	1770±66, 4986±140
VII	2012±80, 5324±136	2110±78, 5342±147	2018±78, 5230±116	2030±74, 5312±132
VIII	1988±83, 5424±141	1985±75, 5373±138	2116±85, 5364±125	1988±78, 5267±131

Significance level, $P < 0.05$.

Table 3. Effect of Diclofenac on C-reactive Protein (CRP) mg/L in Rat After Intraperitoneal Injection of Lipopolysaccharide

Groups	Time (h)			
	0	1	6	24
I	0.8±0.09	1.2±0.11	48.6±4.61	121.3±10.37
II	0.9±0.05	1.8±0.11	51±3.24	119.3±11.85
III	1.02±0.09	1.8±0.1	57.6±4.57	142.6±12.74
IV	0.8±0.08	0.9±0.09	11.6±3.05	35.3±2.02
V	0.28±0.08	1.1±0.1	22±2.65	28.6±3.51
VI	0.653±0.05	0.98±0.1	27±1.73	27.6±3.371
VII	0.69±0.06	0.6±0.11	0.69±0.09	0.62±0.08
VIII	0.69±0.09	0.8±0.09	0.9±0.08	0.8±0.09

Significance level, $P<0.05$.**Table 4.** Effect of Diclofenac on Fibrinogen mg/dL in Rat After Intraperitoneal Injection of Lipopolysaccharide

Groups	Time (h)			
	0	1	6	24
I	310±19	339±24	341±20	610±29
II	304±18	321±19	356±19	596±32
III	296±15	358±21	350±23	588±28
IV	318±14	346±19	348±22	547±31
V	310±13	335±18	360±24	534±29
VI	325±16	332±16	359±21	560±26
VII	328±18	325±18	324±17	320±16
VIII	336±16	330±17	325±15	323±17

Significance level, $P<0.05$.

Gram-negative bacteria that can cause inflammation, septic shock, and death.²⁰ NSAIDs are used for treating inflammatory diseases.^{21,22} The results of this study showed that intraperitoneal injection of LPS could cause alteration in hematological parameters, as the level of WBC in LPS group significantly reduced, and pretreatment with diclofenac could not restore these parameters to normal levels (Table 1). The post-endotoxin leukopenia mainly resulted from a decrease in circulating neutrophil count.^{23,24} Neutrophilia and Lymphopenia were observed in response to the injection of LPS. This, consequently, caused an increase in the leukocyte counts, and leukocytosis, which was in accordance with previous findings.^{25,26} Pretreatment with diclofenac could not inhibit the leukocyte changes (total and diff. count). Previous reports have demonstrated that leukopenia and neutropenia in response to injection of LPS is induced by cytokines which causes margination of neutrophils followed by leukocytosis.²⁷ Neutrophil migration was inhibited, since LPS affected neutrophil chemotaxis. Intraperitoneal injection of LPS alone, in some studies, showed a significant dose- and time-dependent neutrophil migration for mice and horses.^{25,28}

Inflammatory cytokines play an important role in

inflammatory response.²⁹ Interleukins, including IL-1, IL-6, and IL-8 have strong influences on inflammatory responses.³⁰⁻³² CRP and fibrinogen are produced in response to inflammation regulated by IL-1, IL-6, and TNF- α .^{10,11} Our results showed that intraperitoneal injection of LPS could elevate the production of CRP. Pretreatment with diclofenac (at a dose of 2.5 mg/kg) could reduce the production of CRP (Table 3). Moreover, the content of fibrinogen was significantly increased after injection of LPS, and pretreatment with diclofenac (at a dose of 2.5 mg/kg) could not decrease the level of fibrinogen (Table 4). Teeling et al reported that pretreatment with indomethacin and ibuprofen did not reduce the effect of LPS in changing peripheral IL-6, IL-1 β , and TNF- α levels; but dexamethasone altered cytokine production in mice.³³

Conclusion

It can be concluded that diclofenac had different effects on LPS-treated rats. Our results demonstrated that pretreatment with diclofenac could significantly reduce the changes of serum CRP level. The range of these changes should be considered in the evaluation of bacterial toxemia status, and effects of NSAIDs in rats.

Authors' Contributions

Study concept and design: AZ, MRJ and GK. Analysis and interpretation of data: AZ and MRJ. Drafting of the manuscript: AZ. Critical revision of the manuscript for important intellectual content: AZ and MRJ. Statistical analysis: AY and MRJ.

Ethical Approval

This study conformed to the ethical guidelines established by Shahid Chamran University, Ahvaz, Iran.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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