Effects of the Peroxisome Proliferator-Activated Receptors (PPARs) Gamma Agonist on Cecal Ligation and Puncture-Induced Changes in Proinflammatory and Anti Inflammatory Cytokines, Nitric Oxide Production, Renal and Hepatic Injury

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Abstract

Objective: Sepsis is a disorder characterized by systemic inflammatory response caused by an infectious agent and may progress to multiple organ dysfunction and death. Overwhelming inflammatory response is believed to underlie tissue damage and preceding multiple organ dysfunction syndrome. Thus, a therapeutic strategy to inhibit excessive inflammation in sepsis is needed. The nuclear receptor peroxisome proliferator-activated receptors (PPARs) gamma agonists exhibit anti-inflammatory properties in different immune cells. The aim of the present work is to study the effect of administration of PPARs gamma agonist, pioglitazone, on the production of proinflammatory cytokines (tumor necrosis factor-α [TNF-α] and interleukin-6 [IL-6]), anti-inflammatory cytokine (interleukin-10 [IL-10]), nitric oxide, and the changes in systolic blood pressure as well as renal injury (assessed by serum creatinine and histopathological examination) and hepatic injury (assessed by serum alanine aminotransferase activity) induced by cecal ligation and puncture model of sepsis.

Material and Methods: The study was carried out using 36 male albino rats belonging to the local strain. Rats were randomly divided into three groups, each contained 12 rats: Sham operated group (control group, group I), cecal ligation and puncture group (CLP group, group II), and cecal ligation and puncture group with intraperitoneal injection of pioglitazone (CLP+pioglitazone, group III). Pioglitazone was injected intraperitoneally at a dose of 5mg/kg body weight 6 hours after CLP. At the end of the test period (18 hours), before animals were sacrificed, systolic blood pressure was measured and blood samples were withdrawn from retro-orbital plexus, serum was prepared and stored for biochemical analysis. One kidney was removed from each rat for histopathological examination for assessing acute renal injury.

Results: Cecal ligation and puncture (group II) produced, compared to control group, significant (p<0.05) increases in the mean values of serum levels of TNF-α, IL-6, IL-10, and total nitrite, an index of nitric oxide production. Systolic blood pressure significantly (p<0.05) decreased while the mean values of serum creatinine and histopathological score of acute kidney injury significantly (p<0.05) increased. Moreover, mean value serum alanine aminotransferase activity, an index of hepatocyte injury significantly (p<0.05) increased. Intraperitoneal injection of pioglitazone 6 hours after cecal ligation and puncture in rats (group III) at a dose of 5mg/kg, produced, compared to group II, significant (p<0.05) decreases in the mean values of serum levels of TNF-α, IL-6, and total nitrite, significant (p<0.05) increases in the mean values of serum levels of IL-10, and systolic blood pressure. The mean values of serum creatinine, histopathological score of acute kidney injury, and serum alanine aminotransferase activity significantly (p<0.05) decreased compared to group II. Group III demonstrated, compared to control group, significant (p<0.05) increases in the mean values of serum levels of TNF-α, IL-6, IL-10, and total nitrite. Systolic blood pressure significantly (p<0.05) decreased while the mean values of serum creatinine, histopathological score of acute kidney injury, and serum alanine aminotransferase activity significantly (p<0.05) increased.

Conclusion and Recommendations: Administration of pioglitazone, a PPAR gamma agonist, to rats subjected to cecal ligation and punctures decreased mortality, proinflammatory cytokines production, increased anti-inflammatory cytokine production and systolic blood pressure, and decreased renal and hepatic injury compared to rats subjected to cecal ligation and punctures. Further studies will be needed to evaluate the effects of PPAR-gamma at different time intervals following sepsis and to understand the impact of enhancing PPAR-gamma activity before PPAR-gamma agonists can be considered in the treatment of sepsis.

Key Words: Peroxisome proliferator-activated receptors (PPARs) – Nitric oxide production.

Introduction

SEPSIS is a complex clinical entity caused by an individual’s systemic response to an infection and has a wide range of clinical symptoms often leading
to multiple organ dysfunction and/or injury (MOD) and ultimately multiple organ failure (severe sepsis). Sepsis-induced hypotension, despite adequate fluid resuscitation, is termed septic shock [1]. Severe sepsis and septic shock remain the leading causes of death in the non-coronary intensive care units and places a large burden on healthcare resources [2].

Harmful tissue events, including infections, are perceived by macrophages and monocytes, which in turn secrete proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-12 and anti-inflammatory cytokines such as IL-4, IL-10. Appropriate levels of these cytokines are essential for cell mediated microbicidal activity as excessive production can lead to an uncontrolled inflammatory response, multiple organ failure, and ultimately death [3]. The excess release of nitric oxide (NO) resulting from the activation of the inducible isoform of the NO synthase is referred to as a “final mediator” of arterial hypotension during septic shock [4].

Interventions designed to attenuate inflammatory response might be clinically useful. Blocking of the physiological reaction (e.g. blocking TNF-α, TNF-α receptors or IL-1 receptor) has been shown to decrease severity of symptoms in sepsis and mortality in lipopolysaccharide-induced septic shock. However, it does not influence or even increase mortality when subjects, both animals and humans, are exposed to alive microorganisms [5].

The peroxisome proliferator-activated receptors (PPARs) are members of a nuclear receptor family of ligand-dependent transcription factors [6]. The PPAR subfamily comprises three isotypes, PPARα, PPARδ, and PPARγ, which play various roles in lipid and carbohydrate metabolism, cell proliferation and differentiation, and inflammation [7]. Ligands of PPAR-α or PPAR-γ reduce the organ injury and inflammation in animal models of shock [8-10].

The cecal ligation and puncture (CLP) model represents a clinically relevant model of sepsis and has been validated [11].

The aim of the present work is to study the effects of administration of PPARγ agonist (pioglitazone) on CLP-induced changes in serum levels of proinflammatory cytokines (TNF-α and IL-6), anti-inflammatory cytokine (IL-10), serum total nitrite, an index of NO production [12], systolic blood pressure, and the effects of administration of PPARδ agonist on sepsis-induced acute kidney and liver injuries, assessed by serum creatinine level and histopathological scoring of sepsis-induced acute kidney and serum activity of alanine aminotransferase activity.

Material and Methods

The present study was carried out in the Physiology, Biochemistry, Pathology Departments, Faculty of Medicine, Cairo University. The study was conducted between October 2012 and October 2013. 36 male albino rats with body weight ranging from 120-130 grams were included in this study. Rats were placed under ordinary living conditions (e.g. humidity, temperature, and dark/light cycles) in the Animal House. They were kept in wire mesh cages, 12 rats per cage, with free access to rat chow and water. Rats were randomly divided into the following groups:

Group I: Sham operated group (control group).

Group II: (CLP group): Rats of this group were subjected to cecal ligation and puncture (CLP).

Group III: (CLP-pioglitazone treated group): Rats of this group were injected with potassium salt of pioglitazone (Cayman Chemical, Ann Arbor, MI). Pioglitazone was dissolved in phosphate-buffered saline (PBS) and administered intraperitoneally (I.P.) at a dose of 5mg/kg 6 hours after induction of CLP [13]. Group I and group II were injected I.P with equivalent amounts of phosphate-buffered saline 6 hours after the sham operation and CLP operation respectively.

Cecal ligation and puncture:

Rats were fasted 12 hours before the experiment and anesthetized by intraperitoneal injection of thiopentone sodium at a dose of 60mg/kg body weight [14]. Thiopentone sodium (E.I.P.I.C.O., Egypt) vial containing 1 gm was dissolved in 20ml glucose 5%. After shaving abdominal skin, the abdominal skin was disinfected with 70% alcohol. Intra abdominal sepsis was induced by cecal ligation and puncture according to the method described by Wichterman et al. [15], a 2cm ventral midline abdominal incision was made. The cecum was exposed, ligated just below the ileocecal valve with 3-0 silk ligature to avoid intestinal obstruction, and punctured in two locations, 1cm apart on the ante-mesenteric surface of the cecum, with a 22-gauge needle. A small amount of bowel content was extruded through the puncture holes and the cecum was returned to the peritoneal cavity. The abdominal incision was then closed in layers using prolene sutures for the muscles and 3-0 silk for the skin. Immediately following surgery, a single dose of resuscitative fluid (lactated Ringer solution, 50ml/kg body wt) was administered by subcutane-
ous injection [16]. Sham operated rats underwent anesthesia, laparotomy, and wound closure but were not exposed to CLP procedure and given single dose of resuscitative fluid.

After surgery, the rats were returned to the cages and allowed to recover from anesthesia. The rats were allowed free access to water. Rats were sacrificed 18 hours after surgery. This time point was chosen because the animals exhibited significant inactivity and physical evidence of CLP-induced morbidity but did not exhibit mortality until 24-30 hours after CLP [17].

Immediately before animals were sacrificed, systolic blood pressure was measured and blood samples were withdrawn from retro-orbital plexus using capillary tubes, blood was left to clot at room temperature for 30min and then centrifuged for 15min at 2000rpm and serum was then separated and stored frozen (−80°C) for biochemical analysis.

Blood pressure measurement:

Systolic blood pressure was measured by the tail-cuff method using Harvard rat tail blood pressure monitor. The animals were trained to remain calm in a temperature-controlled restrainer prior to the measurement.

Biochemical measurements:

As an index of nitric oxide production [12] total nitrite was determined in serum after conversion of nitrate to nitrite by the enzyme nitrate reductase. Nitrite was then measured by the Griess method using a commercial kit (Total Nitric Oxide Assay) (R&D Systems Inc., Minneapolis, MN, USA) according to the instructions of manufacturers.

Serum levels of rat IL-6, IL-10, and TNF-α were assayed by enzyme-linked immunosorbent assay (QuantiKine, R&D Systems Inc., Minneapolis, MN, USA) according to the instructions of manufacturers.

Serum creatinine was measured using commercial kits supplied by BioMérieux, France. [18], the activity of alanine aminotransferase in serum was estimated using kits supplied by BioMérieux, France [19].

Histopathological scoring of renal injury:

One kidney was removed from each rat for histopathological examination. Paraffin-embedded sections (5 µm) were prepared from the kidneys fixed in 10% phosphate-buffered formalin. The periodic acid-Schiff stain was used for the analysis of morphology by a blinded observer. A semi quantitative score for tubular injury was assigned as described by Wang et al., [20]. At least 10 high-power fields were examined. The percentage of tubules that displayed cellular necrosis, loss of brush border, cast formation, vacuolization, and tubule dilation were scored as follows: 0=None, 1=<10%, 2=11-25%, 3=26-45%, 4=46-75%, and 5=>76%.

Statistical analysis:

Results were expressed as mean ± S.D and subjected to statistical analysis using SPSS window version 9.0. Comparison was done using one-way analysis of variance (ANOVA). $p$-values of <0.05 were considered statistically significant while $p$-values of >0.05 were considered statistically insignificant.

Results

Table (1) demonstrated that at the end of 6 hours after CLP, the mortality rate was 0% in sham operated (group I), group II, and group III. At the end of 18 hours, the mortality rates were 0%, 25%, and 8.3% in group I, group II, and Group III respectively.

Table (1): Mortality rates in cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control group (Group I)</th>
<th>Cecal ligation and puncture (Group II)</th>
<th>Pioglitazone treated group (Group II)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6 hours after</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLP:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>12</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mortality</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>18 hours after</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLP:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>12</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Mortality</td>
<td>0%</td>
<td>25%</td>
<td>8.3%</td>
</tr>
</tbody>
</table>

Compared to control group (group I), cecal ligation and puncture (group II) produced significant ($p<0.05$) increases in the mean values of serum levels of TNF-α, IL-6, and IL-10, and serum total nitrite and significant ($p<0.05$) decrease of systolic blood pressure. The mean values of serum creatinine level, and histopathologic score of acute
kidney injury and serum alanine aminotransferase activity also significantly \((p<0.05)\) increased 18 hours after CLP compared to control group (Table and Fig.).

Intraperitoneal injection of pioglitazone at a dose of 5mg/kg 6 hours after induction of CLP (group III) produced, compared to group II, significant \((p<0.05)\) decreases in the mean values of serum levels of TNF-\(\alpha\), IL-6, IL-10, and serum total nitrite, the mean value of systolic blood pressure. The mean values of serum creatinine level, and histopathologic score of acute kidney injury and serum alanine aminotransferase activity significantly \((p<0.05)\) decreased 18 hours in group III compared to group II (Tables 2-6 and Figs. 1-9).

Compared to control group (group I), intraperitoneal injection of pioglitazone at a dose of 5mg/kg 6 hours after induction of CLP (group III) resulted in significant \((p<0.05)\) increases in the mean values of TNF-\(\alpha\), IL-6, IL-10, and serum total nitrite, the mean value of systolic blood pressure significantly \((p<0.05)\) decreased while the mean values of serum creatinine level, and histopathologic score of acute kidney injury and serum alanine aminotransferase activity significantly \((p<0.05)\) increased 18 hours in group III compared to group I (Tables 2-6 and Figs. 1-9).

### Table (2): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum levels of tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-6 (IL-6), interleukin-10 (IL-10), and total nitrite.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control group Group I</th>
<th>Cecal ligation and puncture Group II</th>
<th>Pioglitazone Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-(\alpha)</td>
<td>29.2±4.7</td>
<td>102.3±10.5 @</td>
<td>63.5±17.6@@#</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>27.6±5.3</td>
<td>143.6±22.1 @</td>
<td>87.1±17.9@@#</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>21.8±5.1</td>
<td>112.6±23.1 @</td>
<td>186.7±14.5@@#</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrite</td>
<td>24.4±5.8</td>
<td>73.2±15.1 @</td>
<td>58.6±6.5@@#</td>
</tr>
<tr>
<td>(umol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ±S.D. 
@ : Significant \((p<0.05)\) changes compared to group I.
# : Significant \((p<0.05)\) changes compared to group II.

### Table (3): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on systolic blood pressure.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control group Group I</th>
<th>Cecal ligation and puncture Group II</th>
<th>Pioglitazone Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121.4±8.2</td>
<td>65.4±7.5@</td>
<td>88.5±11.6@#</td>
</tr>
</tbody>
</table>

Results are expressed as mean ±S.D. 
@ : Significant \((p<0.05)\) changes compared to group I.
# : Significant \((p<0.05)\) changes compared to group II.

### Table (4): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum creatinine levels.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control group Group I</th>
<th>Cecal ligation and puncture Group II</th>
<th>Pioglitazone Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.13±0.05</td>
<td>1.02±0.21 @</td>
<td>0.50±0.14@#</td>
</tr>
</tbody>
</table>

Results are expressed as mean ±S.D. 
@ : Significant \((p<0.05)\) changes compared to group I.
# : Significant \((p<0.05)\) changes compared to group II.

### Table (5): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on Histopathological score of acute kidney injury.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control group Group I</th>
<th>Cecal ligation and puncture Group II</th>
<th>Pioglitazone Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathological score of acute kidney injury</td>
<td>0</td>
<td>4.42±0.67 @</td>
<td>2.75±0.62@#</td>
</tr>
</tbody>
</table>

Results are expressed as mean ±S.D. 
@ : Significant \((p<0.05)\) changes compared to group I.
# : Significant \((p<0.05)\) changes compared to group II.

### Table (6): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum alanine aminotransferase (ALT) activity.

<table>
<thead>
<tr>
<th></th>
<th>Sham operated control group Group I</th>
<th>Cecal ligation and puncture Group II</th>
<th>Pioglitazone Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>18±2.6</td>
<td>66.4±10.5 @</td>
<td>44.8±8.6@#</td>
</tr>
</tbody>
</table>

Results are expressed as mean ±S.D. 
@ : Significant \((p<0.05)\) changes compared to group I.
# : Significant \((p<0.05)\) changes compared to group II.
Fig. (1): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum levels of tumor necrosis factor-α.

@ : Significant ($p<0.05$) changes compared to group I.
# : Significant ($p<0.05$) changes compared to group II.

Fig. (2): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum levels of interleukin-6.

@ : Significant ($p<0.05$) changes compared to group I.
# : Significant ($p<0.05$) changes compared to group II.

Fig. (3): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum levels of interleukin-10.

@ : Significant ($p<0.05$) changes compared to group I.
# : Significant ($p<0.05$) changes compared to group II.

Fig. (4): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum levels of total nitrite.

@ : Significant ($p<0.05$) changes compared to group I.
# : Significant ($p<0.05$) changes compared to group II.

Fig. (5): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on systolic blood pressure.

@ : Significant ($p<0.05$) changes compared to group I.
# : Significant ($p<0.05$) changes compared to group II.

Fig. (6): Effects of cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP on serum levels of creatinine.

@ : Significant ($p<0.05$) changes compared to group I.
# : Significant ($p<0.05$) changes compared to group II.
Histopathological score of acute kidney injury

Fig. (9): Histopathological score of acute kidney injury in cecal ligation and puncture (CLP) and CLP treated with intraperitoneal injection of pioglitazone 5mg/kg 6 hours after CLP. @ : Significant (p<0.05) changes compared to group I. #: Significant (p<0.05) changes compared to group II.

Discussion

Polymicrobial sepsis induced by CLP is the most frequently used experimental model because it closely resembles the clinical conditions of postsurgical peritonitis-induced sepsis and most nearly mimics the pathophysiology of septic human patients [21].

The present study demonstrated significant increases in the mean value of serum levels of TNF-α and IL-6, IL-10, and serum levels of total nitrites, an index of nitric oxide production, significant decrease in systolic blood pressure, and significant increases in serum creatinine level, histopathological scoring of renal injury, and serum alanine aminotransferase activity in cecal ligation and puncture group (group II) compared to control sham operated group (group I).

Main factors which affect prognosis in sepsis are underlying diseases, age, pathogens leading
to sepsis, focus of sepsis, severity of sepsis (sepsis, septic shock, multiple organ failure), appropriateness of antimicrobial treatment, initiation time of antimicrobial treatment, cytokines (as TNF-α, IL-10, IL-6) and coagulation factors [22].

Immune response in sepsis represents the interplay of two contrasting phenomena related to the inflammatory status of the septic patient. The early systemic inflammatory response syndrome (SIRS) is characterized by excessive production of proinflammatory mediators (hyperinflammatory status). This early response is then progressively suppressed by the development of the compensatory anti-inflammatory response (hypoinflammatory status) syndrome (CARS) [23]. This hypothesis was further supplemented by another acronym: The mixed anti inflammatory response syndrome (MARS) representing temporary homeostasis between diminishing SIRS and ascending CARS [24].

Previous theories regarding the pathogenesis of sepsis have implicated proinflammatory cytokines (e.g., TNF-α) as harmful mediators, and numerous strategies aimed at attenuating these mediators have been explored. However, these interventions have proved largely ineffective, this may be because exaggerated hyperinflammatory responses occur less frequently than originally thought [25]. Several theories implicate the later ensuing hypoinflammatory phase of sepsis as being more important in determining the outcome of sepsis [24].

During sepsis, there is a continuous interaction between proinflammatory cytokines (e.g. TNF-α) and anti-inflammatory cytokines (e.g. IL-10) [26].

Meng et al., [27] reported significant increases of plasma levels of IL-6, IL-10, and TNF-α measured 16 hours after CLP. Ertel et al., [28] demonstrated that pro-inflammatory cytokines play a critical role in the initiation and progression of sepsis syndrome and that TNF-α, IL-1β, and IL-6 are important mediators of hemodynamic, metabolic, and immunologic alterations in the host during sepsis. Circulating concentrations of TNF-α, IL-1β, and IL-6 increase significantly in the early, hyperdynamic stage of sepsis and remain elevated in the late, hypodynamic stage of sepsis.

Overproduction of proinflammatory mediators plays an important role in the pathogenesis of multiple organ dysfunction syndrome in sepsis [29]. Higher levels of TNF-α are correlated with poor outcomes in septic patients, suggesting that TNF-α synthesis must be controlled [30]. Proinflammatory mediators TNF-α, IL-6, and nitric oxide are elevated in patients with severe sepsis [31,32]. IL-10 is a pleiotropic cytokine with potent anti-inflammatory properties; however, over-production of IL-10 correlate with severity of the septic insult and higher mortality [33].

TNF-α is considered to be a “master regulator” of inflammatory cytokine production. TNF-α is not only derived predominantly from activated immune cells (macrophages) but also from non immune cells (fibroblasts) in response to invasive, infectious, or inflammatory stimuli [34]. The release of TNF-α from macrophages begins within 30 minutes after the inciting event, following gene transcription and RNA translation, which established this mediator to be an early regulator of the immune response. TNF-α acts via specific two transmembrane receptors [35], leading to the activation of immune cells and the release of an array of downstream immunoregulatory mediators. TNF-α acts on different target cells, such as macrophages, endothelial cells, and neutrophils. TNF-α leads to an enhanced production of macrophages from progenitor cells [36], promotes the activation and differentiation of macrophages [37], and prolongs their survival [38]. All these effects enhance proinflammatory responses in sepsis. In endothelial cells, TNF-α enhances the expression of adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, and chemokines [39]. TNF-α also increases integrin adhesiveness in neutrophils and promotes their extravasation into tissues.

IL-6 is produced by a wide variety of cells, especially macrophages, dendritic cells, lymphocytes, endothelial cells, fibroblasts, and smooth muscle cells in response to stimulation with lipopolysaccharide, IL-1, and TNF-α [40] and peaks subsequent to TNF-α and IL-1 concentrations [41]. IL-6 has a variety of biological effects, including the activation of B and T lymphocytes [42]. A key function of IL-6 is the induction of fever [43] and the mediation of the acute phase response [44], a systemic reaction to an inflammatory stimulus that is characterized by fever, leukocytosis, and the release of hepatic acute phase proteins such as C-reactive protein, complement components, fibrinogen, and ferritin [45].

IL-10 is produced by many types of immune cells, such as monocytes, macrophages, B and T lymphocytes, and natural killer cells [46]. IL-10 suppresses the production of proinflammatory mediators, such as TNF-α, IL-1, IL-6, and interferon gamma (IFN-γ) in immune cells [47]. Additionally, IL-10 stimulates the production of interleukin-1 receptor antagonist and soluble tumor
necroptosis factor receptors, thereby neutralizing the proinflammatory actions of IL-1 and TNF-α [48]. Inhibition of IL-10 12 hours after CLP markedly improved survival [49]. However, the administration of neutralizing IL-10 antibodies at the time of CLP partially exacerbated mortality [50]. These results suggest that IL-10 might regulate the transition from early reversible sepsis to late irreversible septic shock [51].

Toll-like receptors (TLRs) play a critical role in the induction of innate immune and inflammatory responses. TLRs recognize pathogen associated molecular patterns (PAMPs) and transduce a signal into the cell [52]. TLR-mediated signaling predominately activates nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) which is an important transcription factor controlling the expression of inflammatory cytokine genes. TLR4-mediated NF-κB activation plays an important role in mediating the pathophysiology of coecal ligation and puncture (CLP)-induced sepsis/septic shock [53].

NF-κB regulates the expressions of proinflammatory cytokines and chemokines [54]. Myeloid differentiation primary response 88 (MyD88) is a central adaptor protein for the majority of TLRs, acting as a link between the receptors and downstream kinases [55]. In resting cells, NF-κB is sequestered in the cytoplasm in an inactive state bound to its inhibitor protein, IκB. Upon stimulation, IκB-α is phosphorylated and degraded, resulting in the release and translocation of NF-κB to nuclei [56]. At least five members of the NF-κB family (p50, p65, Rel-B, C-Rel and p52) have been identified in mammals [57]. Phosphorylation of the p65 subunit of NF-κB may contribute to complete p65 activation and subsequent DNA binding which activates the transcription of target genes, such as cytokines, and inducible nitric oxide synthase [58].

In the present work, CLP induced significant decrease in systolic blood pressure compared to sham operated control group (group I).

Vasodilatation of the peripheral (systemic) resistance vessels occurs in severe sepsis, the term “vasoplegia” is often applied to the vasculature, suggesting that rhythmic vasomotion is paralysed in severe sepsis [59].

The hyperdynamic state of sepsis persists from 2 to 10h and the hypodynamic state occurs 16 to 20h after CLP, depending on the lesion in the cecum. Mean arterial blood pressure decreased 10h after CLP, which is an indicator for the occurrence of hypodynamic sepsis [15].

Sepsis is characterized by systemic inflammation [60], decreased peripheral vascular resistance [61], microvascular leak [62], and decreased cardiac output [61]. The combined effect of these alterations is low blood pressure (hypotension), a major clinical feature of sepsis. This hypotension results in under perfusion of end organs that leads to their functional failure and too often patient death [61].

Excess release of nitric oxide (NO) resulting from the activation of the inducible isoform of the NO synthase (iNOS) is referred to as a “final mediator” of arterial hypotension during septic shock [4]. The present work demonstrated significant increase in serum level of total nitrite (nitrates/nitrites), an index of NO production [12]. Large amounts of NO are produced by iNOS in response to bacterial endotoxin or inflammatory cytokines in many tissues, including vascular endothelium, vascular smooth muscle, and myocardium [63]. This enhanced NO formation contributes to severe arterial hypotension, vascular hyporeactivity to vasoconstrictors, and myocardial dysfunction [64].

NO is synthesized from L-arginine by the enzyme NO synthase (NOS), which has constitutive and inducible isoforms encoded by distinct genes. The inducible NOS isoform (iNOS) is present in macrophages, hepatocytes, and vascular smooth muscle cells; is regulated at the transcriptional level; and releases large amounts of NO in a sustained fashion after stimulation with mediators associated with sepsis, such as endotoxin, TNF-α, interleukin-1, interleukin-2, and interferon-γ [65]. Overproduction of NO by activation of iNOS has been well documented both in animal models of sepsis and in septic patients [66,67].

NO binds with high affinity to ferrous heme bound to the β-subunit of soluble guanylate cyclase (sGC), resulting in pronounced stimulation of cyclic guanosine monophosphate (cGMP) formation [68]. The mechanisms proposed for cGMP-mediated vasodilation include: 1- Inhibition of inositol-1,4,5-triphosphate generation; 2- Enhanced cytosolic Ca2+ extrusion; 3- Diphosphorylation of myosin light-chain kinase; 4- Ca2+ Influx inhibition; 5- Protein kinase G activation; 6- Stimulation of membrane Ca2+ ATP ase; and 7- Potassium channels opening [69]. Moreover, vascular resistance to the pressor response to norepinephrine [70] and angiotensin II [71] occurs during sepsis and is attributable in part to the potent vasodilatory effect of nitric oxide [72].
An increase in plasma concentrations of hydrogen ions and lactate and a decrease in ATP in vascular smooth-muscle cells during septic shock activate the ATP-sensitive potassium channels (\( K_{\text{ATP}} \) channels) [73,74]. The resultant potassium efflux through the \( K_{\text{ATP}} \) channels causes hyperpolarization of the vascular smooth-muscle cells with closure of the voltage-gated calcium channels in the membrane. The high endogenous levels of vasoactive hormones during sepsis may be associated with down-regulation of their receptors, which would result in a lessening of their effects on the vasculature [75].

Inducible nitric oxide synthase (iNOS) is an important target for NF-\( \kappa \)B in the cellular response to inflammatory stimuli [76]. Cytokines and products of the bacterial wall elicit the expression of iNOS in a variety of tissues and cell types. NO contributes to the regulation of the inflammatory process through the modulation of enzyme activity and gene expression in an autocrine and paracrine fashion [77].

The results of the present study demonstrated significant increase in the mean value of serum creatinine level in CLP group (group II) compared to control group. Histopathological examination revealed evidence of acute tubular necrosis in rats subjected to CLP.

Acute kidney injury (AKI) is as an abrupt reduction in glomerular filtration and the first diagnostic test of kidney function loss is clinically evidenced by an increase in serum creatinine levels, criteria that are imprecise and tardy [78]. The ADQI (Acute Dialysis Quality) group proposed the AKI severity classification: RIFLE (Risk, Injury, Failure, Loss, End) [79]. Another research group, AKIN (Acute Kidney Injury Network), changed the RIFLE classification and defined AKI as an increase in serum creatinine levels by 0.3mg/dl or more or a percentage increase of 1.5 times the baseline value or more in the last 48 hours. Additionally, the decreased urine output criteria came to exert a marker function. This diagnostic consensus is the most accepted [80].

In septic acute kidney injury (AKI), serum creatinine doesn’t accurately reflect the glomerular filtration rate, because the patient is not in steady state condition. Furthermore serum creatinine is also influenced by tubular creatinine secretion and non renal factors such as muscle mass, liver function and non-renal gastrointestinal elimination [81]. Doi et al. [82] reported, in their animal study, that sepsis dramatically reduces the production of creatinine without changes in body weight, hematocrit and extracellular fluid volume.

AKI occurs in 20-50% of septic patients [75] and approximately doubles the mortality rate to near 70% [83]. Rodent models of sepsis-induced AKI suggest that intrarenal microcirculatory failure is a key event leading to the development of septic AKI [84,85].

The pathophysiology of organ dysfunction during septic shock is multifactorial and not well understood. Although systemic hemodynamic decline during sepsis can contribute to organ hypoperfusion, there is a growing appreciation of the importance of microcirculatory failure in the development of organ injury. Micro vascular dysfunction is now recognized as a strong predictor of death among patients with severe sepsis [86].

Excessive inflammatory response and cytokine release occurs in sepsis, leading to increased superoxide anion and NO production [87]. Overproduction of superoxide anion causes the inflammatory cascade at the vessel wall, resulting in over expression of P-selectin and intracellular adhesion molecule-1 [88]. Strong interaction between leukocytes and endothelial cells is implicated in vascular dysfunction and tissue injury [89]. In addition, superoxide anion reacts rapidly with NO to form the peroxynitrite [90]. Peroxynitrite formation in tissues has been demonstrated from different animals challenged CLP [91]. The most important effect of peroxynitrite in sepsis is exerted on mitochondria to cause single-stranded DNA breaks. DNA injury activates poly (ADP-ribose) polymerase (PARP) to destroy nicotinamide adenine dinucleotide, causing energy depletions and cellular injury [92]. One crucial factor underlying the pathogenesis of multiple organ dysfunction syndrome in sepsis may be due to the derangements in cellular energy metabolism by peroxynitrite [93]. PARP activation causes organ damage in CLP-induced septic shock.

In the kidneys of animals with sepsis, there is marked upregulation of TLR4 [94], which is known to stimulate activation of the proinflammatory transcription factor NF-\( \kappa \)B [95]. The activation of NF-\( \kappa \)B plays a central role in the pathophysiology of septic shock [96] inducing systemic cytokine production and thus triggering mechanisms that depress renal function in response to inflammatory diseases [97].

The initial inflammatory response during sepsis is characterized by a robust increase in proinflammatory cytokines, such as TNF-\( \alpha \) [98], which trigger
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an early cascade of downstream events including upregulation of inducible nitric oxide synthase (iNOS) [99], the generation of reactive oxygen species (ROS) [100] and reactive nitrogen species (RNS) [101], and increased endothelial permeability and microvascular leakage [100]. Paradoxically, activation of homeostatic mechanisms to raise systemic pressure during septic shock such as activation of the renin-angiotensin system can increase renal vascular resistance and intensify the development of AKI [102]. While the effects of sepsis on renal blood flow (RBF) in humans are still controversial, in rodent models of severe sepsis a fall in RBF [103] and renal microcirculatory dysfunction [99] precede the onset of AKI. Agents which scavenge oxidants and improve the renal microcirculation improve renal function in a coecal ligation and puncture (CLP) model of murine sepsis [100].

The reduction in renal blood flow and hypoperfusion result in low oxygen supply, which induces the tubular epithelial cell injury, apoptosis and acute tubular necrosis in prolonged hypoperfusion [104]. Other factors associated with the pathophysiology of sepsis, such as absolute hypovolemia, vasoplegia and capillary extravasations with interstitial edema contribute to the reduced oxygen transportation [105]. Renal failure happens when the organ function is compromised, with signs of water and electrolyte homeostasis imbalance and accumulation by reduction of the metabolic excretion of nitrogenous products, including serum urea and creatinine [104,106].

Serum alanine aminotransferase activity, used to determine the degree of liver damage [107], significantly increased in CLP group compared to control group.

Hepatic dysfunction occurs during early sepsis [108]. Sepsis-induced liver organ failure is associated with increased mortality [109]. Over activation of proinflammatory mediators plays an important role in sepsis-induced liver injury [110]. Neutrophil infiltration is a critical step in the development of organ dysfunction. Myeloperoxidase activities in organs can represent tissue injury resulting from neutrophil infiltration [111].

Sepsis-induced liver dysfunction can be divided into primary and secondary hepatic dysfunctions. Primary hepatic dysfunction is most likely linked to systemic or microcirculatory disturbances. This often leads to disseminated intravascular coagulation, reduced hepatic lactate and amino acid clearances, decreased gluconeogenesis and glycogenolysis with subsequent hypoglycemia. The acute cellular and mitochondrial damage of hepatocytes is manifested by a leak of aminotransferase enzymes. Secondary hepatic dysfunction is thought to be due to spillover of bacteria and/or endotoxin and the subsequent activation of inflammatory cytokines and mediators [112].

In sepsis and systemic inflammatory response syndrome, the liver is a key organ that plays two opposing roles: it is both a source of inflammatory mediators and a target organ for the effects of the inflammatory mediators. The liver is pivotal in modulating the systemic response to severe infection as it contains the largest mass of macrophages (Kupffer cells) in the body that are able to clear the endotoxin and bacteria that initiate the systemic inflammatory response [113]. At the same time, Kupffer cells as well as hepatocytes may get activated by spillover of bacteria or endotoxin and become sources for soluble mediators of systemic inflammatory response syndrome [114].

Liver microcirculation disturbances are a cause of hepatic failure in sepsis. Increased leukocyte-endothelial interaction, platelet adherence and impaired microperfusion cause hepatocellular damage [115].

Sepsis therapies are mostly supportive in nature and all specific experimental therapeutic approaches, except early administration of antibiotics and early goal-directed therapy, have failed to be translated successfully into the clinical setting [116]. Thus, new pharmacological strategies are urgently needed to improve the treatment of this condition [117].

The development of an appropriate pharmacologic intervention for the treatment of sepsis-induced multiple organ dysfunctions is proving difficult because of the complexity of the disorder. However, targeting the excessive inflammatory response is thought to be of significant importance in preventing multiple organ dysfunction [118].

In the present study, treating rats subjected to CLP with pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, at a dose of 5mg/kg 6 hours after CLP (group III), produced significant decreases in the mean values of serum levels of TNF-α, IL-6, significant increase in the mean value of serum level of IL-10, significant decrease in the mean value of serum level of total nitrite while systolic blood pressure significantly increased. The mean value of serum levels of creatinine, histopathological scoring of acute kidney injury and serum alanine aminotransferase activity
The peroxisome proliferator-activated receptors (PPARs) are members of a nuclear receptor family of ligand-dependent transcription factors [6]. The PPAR superfamily comprises three isotypes, PPAR-α, PPAR-β/δ, and PPAR-γ, which play various roles in lipid and carbohydrate metabolism, cell proliferation and differentiation, and inflammation [7]. PPARs are activated by a diverse group of fatty acids and their derivatives, a variety of eicosanoids, and a number of selective synthetic drugs, such as the fibrate class for PPAR-α, thiazolidinediones for PPAR-γ, and L-165041 for PPAR-β/δ [119].

A wide range of synthetic PPAR-γ ligands have been developed. The most widely used belong to the thiazolidinedione (TZD) or glitazone class of anti-diabetic drugs used in the treatment of type 2 diabetes. These include rosiglitazone, pioglitazone, cigitazone, and troglitazone. Troglitazone was withdrawn from the market following the emergence of a serious hepatotoxicity in some patients. The two currently available PPAR-γ agonists, rosiglitazone and pioglitazone, are currently used alone or in combination with other oral anti-diabetic agents for type 2 diabetes patients [120].

The PPAR-γ protein is a complex formed of several domains such as a DNA-binding domain (DBD) and a ligand binding domain (LBD), it also has a D-domain, which links the DBD and LBD [121]. In the absence of a ligand, the PPAR will be associated with a corepressor complex to repress the target genes. Whereas, when a specific ligand binds to the LBD, the PPAR complex dimerizes with retinoic acid-X-receptor (RXR) leading to release of the corepressor [122]. The PPAR/RXR heterodimer translocates to the nucleus and binds to the promoters of several genes that contain a peroxisome proliferator response element (PPRE) to either initiate or suppress the transcription of the target genes [123].

PPARγ are also capable of regulating gene expression independently of binding to PPREs. The activity of a number of transcription factors, for example NF-κB, AP-1 (activator protein-1), and STAT-1 (signal transducer and activator of transcription), can be inhibited by PPARs either via direct interaction or by competition for limited supplies of coactivators [124]. The proinflammatory transcription factor NF-κB plays a central role in the immune and inflammatory responses [125]. It is the major target for PPAR-γ to suppress inflammation [126]. This function is important in regard to the anti-inflammatory properties of PPARs since proinflammatory gene expression is mainly affected by this direct repression of transcription factors [127].

PPARγ ligand reduces the DNA-binding activity of nuclear factor (NF)-κB. This finding suggests that the anti-inflammatory properties of the PPARγ ligand may be related to the regulation of NF-κB activation [8]. Activation of NF-κB is a pivotal step in the regulation of the expressions of pro-inflammatory genes, including tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS), and cyclooxygenase (COX)-2 [128].

The timing of administering PPAR-gamma agonists in sepsis could be crucial for their effect on attenuating the septic response and improving survival. Most of the studies using thiazolidinediones in experimental sepsis models have given the drugs either before or shortly after the onset of sepsis [8,13,129].

Several studies provide evidence for amelioration through treatment with 15-deoxy-delta 12,14-prostaglandin J2 (15d-PGJ2), a natural PPAR-gamma agonist [130], of endotoxic shock induced in rodents by bacterial products such as LPS, and wall fragments of Gram-positive and Gram-negative bacteria [120,131]. The 15d-PGJ2 likely counteracts the inflammatory response by activating PPARγ, repressing NF-κB, and enhancing the heat shock response [132]. Evidence for direct involvement of PPARγ in organ protection is provided by a reduction of the beneficial action of 15d-PGJ2 in the presence of the PPARγ antagonist GW9662 [120]. PPAR-gamma agonists can reduce systemic mediators in experimental models of sepsis [8] and endotoxin-induced acute lung injury [129].

PPARγ is expressed in and mediates important immunoregulatory functions in conventional T cells, macrophages, and dendritic cells [133,134]. PPAR-γ is expressed in human and murine monocytes/macrophages and there is a link between the state of monocyte/macrophage differentiation or activation and PPAR-γ expression. In the mouse, PPAR-γ is expressed at low levels in non-activated macrophages, whereas much higher levels are expressed in activated peritoneal macrophages [135]. In human peripheral blood monocytes, a similar relationship between the state of differentiation and activation and PPAR-γ expression has been described [136]. In addition to PPAR-γ expression being up-regulated after macrophage activation, PPAR-γ activation
can itself lead to differentiation of monocytes along the macrophage lineage [137].

PPAR-γ agonists suppress the production of the inflammatory cytokines as IL-1β, IL-6 and TNF-α in stimulated human peripheral blood monocytes [138]. Ricote et al., [135] demonstrated that PPAR-γ ligands inhibit the expression of iNOS, gelatinase B and scavenger receptor A genes, in part by antagonizing the activities of the transcription factors AP-1, STAT-1 and NF-κB.

TZDs can repress AP-1- and NF-κB-dependent expression of inflammatory genes, and this may occur in large part via PPAR-γ-mediated stabilization of the nuclear receptor corepressor (NCoR) complex [139]. TZDs may also drive expression of a variety of anti-inflammatory genes, such as adipopectin [140], heme oxygenase-1 [141], and IL-10 [142]. The ability of PPAR-γ agonists to repress AP-1- and NF-κB-dependent expression of inflammatory genes and the ability to drive expression of a variety of anti-inflammatory genes can explain the significant decreases in mean values of serum levels of TNF-α and IL-6 and significant increase in the mean value of serum level of IL-10 in CLP group treated with pioglitazone at a dose of 5mg/kg 6 hours after induction of CLP (group III) compared to CLP group (group II).

Ferreira et al., [143] reported reduced proinflammatory cytokine production and high IL-10 levels in pioglitazone-treated septic mice. These effects were associated with a decrease in STAT-1-dependent expression of MyD88. IL-10 receptor blockade abolished PPAR-gamma-mediated inhibition of MyD88 expression. These data demonstrate that the primary mechanism by which pioglitazone protects against polymicrobial sepsis is through the impairment of MyD88 responses. Ferreira et al., [143] also reported that pioglitazone, which has been approved by the U.S. Food and Drug Administration, improves sepsis outcome, improves microbial clearance, and enhances neutrophil recruitment to the site of infection. This appears to represent a novel regulatory program. In this regard, pioglitazone provides advantages as a therapeutic tool, because it improves different aspects of host defense during sepsis, ultimately enhancing survival.

PPAR gamma agonists are a double-edged sword in sepsis. On the one hand, PPAR gamma agonist inhibits proinflammatory gene expression. The expressions of the affected genes, such as those for inducible nitric oxide synthase, TNF-α, or IL-1β are repressed. Therefore, PPAR gamma is suggested to be beneficial in hyper-inflammatory diseases, such as sepsis. In animal models of sepsis, treatment with PPAR gamma agonist attenuated inflammation and organs dysfunctions, accompanied by their improved survival rate. On the other hand, PPAR gamma agonists provoke apoptosis, which in the hyper-inflammatory phase of sepsis might be helpful because the number of immune cells, such as monocytes, macrophages, and neutrophils, involved in secreting high amounts of proinflammatory mediators will be reduced. In contrast, during the anti-inflammatory phase, cell death of immune cells, especially of T lymphocytes, is supposed to be deleterious. Under these circumstances, a second infection cannot be adequately answered. Therefore the role of PPAR gamma is still ambiguous [144].

In the present work, serum levels of total nitrite were measured to determine whether PPAR γ agonist affects NO production in sepsis. The results of the present work demonstrated that PPAR γ agonist produced significant decreases in the mean value of serum total nitrite levels is CLP-induced sepsis treated with pioglitazone compared to CLP group. NO is generated from inducible NO-synthase (iNOS) through the activation of NF-κB [145]. PPARγ activation inhibits NO overproduction, IL-6, and TNF-α expression and suppresses iNOS induction by repression of NF-κB and activator protein-1 activation [146,147]. The decreased production of NO, as evident by significant decrease in the mean value of serum total nitrite levels in CLP-induced sepsis, treated with pioglitazone, compared to CLP group can explain the significant increase in systolic blood pressure in group III compared to CLP group. Treatment with 15d-PGJ2 or ciglitazone, one of the TZDs, following CLP ameliorated hypotension and survival, blunted cytokine production and reduced neutrophil infiltration in lung, colon, and liver. These beneficial effects of PPAR-γ ligands were associated with the reduction of IκB kinase complex, and reduction of NF-κB and AP-1 pathways [8].

In the present work, administration of pioglitazone to rats subjected to CLP decreased renal injury as evident by significant decrease of serum creatinine and histopathological score of sepsis-induced acute kidney injury compared to group II.

In renal tissue, PPARγ are predominantly expressed in collecting ducts and, to a lesser extent, in glomeruli, mesangial cells, proximal tubules and the renal microvasculature [148].
In sepsis-induced acute kidney injury, TNF-α magnifies the responses mediated by other cytokines and free radical generation through activation of the NF-κB signalling pathway [149]. Promoting the activity of PPAR-γ could decrease TNF-α during sepsis by inhibiting NF-κB activation [150].

In the different non-diabetic animal models with nephropathy such as renal ischemia-reperfusion induced injury in rats [151], cyclosporine A-induced renal injury in rats [152], renal dysfunction induced by sepsis in mice [150] and the 5/6-nephrectomy in rats [153], prior administration of PPARγ agonists constantly generated a protective effect, as demonstrated by less interstitial inflammatory cell infiltration, interstitial fibrosis and proinflammatory mediators compared with untreated controls [150-153]. All those beneficial effects were independent of the PPAR γ agonist’s action on lipid and glucose homeostasis.

Lee et al., [150] reported that rosiglitazone significantly decreased serum tumour necrosis factor-α and interleukin-1β levels during sepsis. The levels of blood urea nitrogen and creatinine were significantly lower in mice pre-treated with rosiglitazone than that in lipopolysaccharide-treated mice. Rosiglitazone reduced the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in tubular epithelial cells and interstitium of lipopolysaccharide-treated mice. Pre-treatment with rosiglitazone reduced the infiltration of macrophages/monocytes in renal tissue. In cultured tubular epithelial cells, rosiglitazone significantly decreased the expression of ICAM-1 and VCAM-1 induced by TNF-α or IL-1β, inhibited the degradation of inhibitor κBα (IκBα) and blocked the activation of the p65 subunit of nuclear factor (NF)-κB.

In the present work, administration of PPAR γ agonist (pioglitazone) decreased hepatic injury in rats subjected to CLP as evident by decreased serum alanine aminotransferase activity compared to group II.

Zhou et al., [154] found that, by using cecal ligation and puncture and administration of lipopolysaccharide, hepatic PPAR-γ expression in Kupffer cells is down regulated, possibly by increased plasma TNF-α. Decreased PPAR-γ expression, in turn, increased the production of proinflammatory cytokines and tissue injury. Higuchi et al., [155] revealed down-regulation of hepatic PPARγ in animals with a double-hit model of hemorrhage and sepsis, which mediated hepatocellular injury and production of pro-inflammatory mediators. Treatment with the natural ligand, 15d-PGJ2, and the synthetic ligand, ciglitazone, for PPARγ was reported to attenuate cytokine production and neutrophil infiltration in the lungs, colon, and liver of septic rats [8].

Despite the ability of PPARγ to decrease mortality rate, serum levels of TNF-α, IL-6, serum total nitrite, serum creatinine, serum alanine aminotransferase activity, and histopathological score of acute kidney injury and the ability to increase serum IL-10 and systolic blood pressure in rats subjected to CLP compared to rats subjected to CLP alone. The results of the present work demonstrated an increased mortality, significant increases of serum levels of TNF-α, IL-6, IL-10, serum total nitrite, serum creatinine, serum alanine aminotransferase activity, and histopathological score of acute kidney injury, and significant decrease of systolic blood pressure in rats subjected to CLP with pioglitazone treatment compared to sham operated group. This can be explained by the complexity of inflammatory process, disturbed internal environments, timing and dose of PPAR-γ agonist administration, lack antimicrobial treatment, and contribution of other inflammatory mediators not controlled by PPARγ agonist.

Summary, Conclusion and Recommendations:

Administration of pioglitazone, a PPAR gamma agonist, to rats subjected to cecal ligation and punctures decreased mortality, proinflammatory cytokines production, increased anti-inflammatory cytokine production and systolic blood pressure, and decreased hepatic and renal injury. However, concern has been raised that role of PPAR-gamma in sepsis may be that of a “double-edged sword,” by provoking apoptosis of immune cells, thereby rendering a host vulnerable to a second infection in the anti-inflammatory phase following a septic insult. Further studies are needed to evaluate the effects of PPAR-gamma at different time intervals following sepsis and to understand the impact of enhancing PPAR-gamma activity before PPAR-gamma agonists can be safely considered in the treatment of patients with sepsis.

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