Original Article

PPAR-γ agonist improve endothelium injury by reducing oxidative stress and inflammation in septic rats

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Abstract: Objective: This study was conducted to verify the effects and mechanisms of peroxisome proliferators-activated receptor-γ (PPAR-γ) agonist on endothelium injury in septic rats induced by lipopolysaccharide (LPS). Method: Total 40 Sprague-Dawley (SD) rats were randomly divided into 4 groups, namely control group, LPS stimulation group, rosiglitazone (ROSI) pretreatment group, and Selective Antagonist 2-Chloro-5-nitro-benzamine (GW9662) pretreatment group. Immunohistochemistry was performed to detect the expression of PPAR-γ in different groups. The blood samples were taken from each group after 4 hours to detect endothelium injury markers (VCAM-1, ICAM-1, Ang-2, TM, AT-III, TF and vWF), the amount of circulating endothelial cell (CEC), oxidative stress-related factors (NO, eNOS and SOD) and inflammation-related factors (TNF-α, IL-6, IL-10, TGF-β, MMP-9, CRP and PCT). Result: The expression level of PPAR-γ was decreased after LPS stimulation as compared with that in control group, which was then increased in ROSI pretreatment group and decreased in GW9662 pretreatment group. Consistently, endothelium injury markers (VCAM-1, ICAM-1, Ang-2, TM, AT-III, TF and vWF), the amount of circulating endothelial cell (CEC), oxidative stress-related factors (NO, eNOS and SOD) and inflammation-related factors (TNF-α, IL-6, IL-10, TGF-β, MMP-9, CRP and PCT) were significantly increased in LPS stimulation group compared with control group (P < 0.01). Additionally, all these factors were significantly decreased in ROSI pretreatment group (P < 0.01) whereas almost unchanged in GW9662 as compared with LPS Group (P > 0.01). Conclusion: PPAR-γ agonist enhanced endothelial functions and reduced endothelial injury by reducing oxidative stress and inflammation in septic rats.

Keywords: Peroxisome proliferator activated receptor-γ, sepsis, endothelial injury, inflammatory response, oxidative stress

Introduction

Sepsis is a leading cause of death among hospitalized patients in noncoronary intensive care units, which results from a damaging or overwhelming complicated host response including activation of several cell types, coagulation and inflammatory factors [1, 2]. Although tremendous progress has been made in understanding the association between sepsis-associated mortality and host response and outcomes have improved, the overall mortality of sepsis remains at 31% overall [3]. Thus, identification of new targets and mechanisms are still necessary for the treatment of sepsis.

Recently, it is well established that the endothelium function plays a pivotal role in mediating the sepsis phenotype [4, 5]. Endothelial cells not only provide an anticoagulant barrier between blood and tissue, but also play an important role in hemostasis, immune, inflammatory responses, angiogenesis and others [6]. Moreover, the activation, dysfunction and injury of endothelium are considered to be the main hallmarks of sepsis [5, 7]. Notably, it has been widely accepted that the dysfunctional or damaged endothelial cells can release a large variety of inflammatory factors, leading to the amplification of inflammatory response [8, 9]. Meanwhile, antioxidant defenses are overwhelmed during sepsis, and reactive oxygen species (ROS) cause cellular damage, contributing to the dysfunction and injury of endothelium [10, 11]. Thus, inflammatory response and oxidative stress may play important roles in leading to endothelial dysfunction or damage.
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Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of ligand-activated transcription factors which belongs to the nuclear receptor superfamily. It plays an important role in controlling the inflammatory response and improving the function of endothelial cells [12]. Numerous studies have confirmed that PPAR-γ has the potential efficacy as a therapeutic approach in sepsis and inflammation [13]. PPAR-γ ligands may attenuate the inflammatory response in sepsis via the regulation of the NF-κB and AP-1 pathways [14]. Rosiglitazone (ROSI), a PPAR-γ ligand, has been identified as specific PPAR-γ agonist that can inhibit production of several inflammatory cytokines [15, 16]. Moreover, Millioni et al. found that PPAR-γ activation could reduce the apoptosis rate of endothelial cells by 30%-50% in high glucose conditions and increase protein expression of endothelial cells for maintaining the endothelial cell function [17]. Thus, PPAR-γ may be a key inflammatory molecule that plays an important role in the protection of endothelial cells in sepsis.

In our current experiment, the septic model was established via lipopolysaccharide (LPS) venous injection. After treated with PPAR-γ agonist-ROSI and PPAR-γ selective antagonist 2-Chloro-5-nitro-benzamine (GW9662) [18], the levels of endothelium injury markers, the amount of circulating endothelial cell (CEC), oxidative stress-related factors and inflammation-related factors were detected in the septic model rats to explore whether PPAR-γ and its ligand could deduce endothelial injury and whether such intervention effects would be reversed by GW9662. Our study might verify the effect of PPAR-γ agonist on endothelium injury in septic rats induced by LPS, as well as to provide theoretical and experimental foundation for clinical prevention and treatment of endothelial injury in sepsis.

Materials and methods

Reagents and animal groups

PPAR-γ agonist-ROSI and selective antagonist GW9662 were purchased from GlaxoSmithKline (GlaxoSmithKline UK Ltd., Avandia, UK) and dissolved in 10% dimethylsulfoxide (DMSO). LPS (E.coli O55:BS) were purchased from Sigma (St. Louis, MO, USA). The specific pathogen-free (SPF) male Sprague-Dawley (SD) rats weighing of 250 ± 30 g were obtained from the center of experimental animals (Shandong Luye Pharmaceutical Co., Ltd., China) and all the animals were fed standard diet and free access to tap water before the experiment.

At the beginning of experiment, 3% pentobarbital sodium (according to the body weight of 50 mg/kg) was injected into the enterocoeila of rats for anesthesia. Then these 40 healthy male SD rats were randomly divided into 4 groups with 10 rats per group. Control group: the rats were injected with 1 mL/kg 10% DMSO and then injected with 2 mL/kg normal saline (NS) 30 min later; LPS stimulation group: the rats were injected with 1 mL/kg 10% DMSO and then injected with 6 mg/kg LPS 30 min later; ROSI pretreatment group (ROSI + LPS): the rats were injected with 0.3 mg/kg ROsi and then injected with 6 mg/kg LPS 30 min later; GW9662 pretreatment group (GW9662 + ROsi + LPS): the rats were injected with 0.3 mg/kg GW9662, 0.3 mg/kg ROsi at 15 min later, and 6 mg/kg LPS at 30 min later.

After 4 hours of each group’s experiment, 4 mL blood samples were extracted from abdominal aorta of each rat using intubation for the subsequent detection and analyses.

Immunohistochemical analysis

To detect the expression of PPAR-γ in ROSI pretreatment group and GW9662 pretreatment group, immunohistochemical analysis was performed using immunohistochemical kit (BO-STER Co., Ltd., China) according to the manufacturer’s instructions. Briefly, samples were fixed with 30% H2O2 and 100% cold methanol for 30 min. Then the samples were incubated with rabbit-anti-rat PPAR-γ (1:50) at 4°C overnight, followed by the non-biotinylated secondary antibody at 37°C for 20 min. Subsequently, the samples were incubated with horseradish peroxidase (HRP)-streptavidin, stained with 3,3’-diaminobenzidine (DBA) and re-stained with hematoxylin. Finally, the samples were observed using a microscope (Olympus, Japan) and 5 random images were taken for each sample.

Enzyme-linked immunosorbent assay (ELISA)

After the blood samples were centrifuged, the serum was taken to detect the markers of endothelial injury and inflammation for each
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Figure 1. The expression of PPAR-γ in 4 groups by immunohistochemical analysis. A: Control group; B: LPS stimulation group; C: Rosiglitazone (ROSI) pretreatment group; D: Selective Antagonist 2-Chloro-5-nitro-benzamine (GW9662) pretreatment group.

The measurement of ROS was conducted using a kit developed by the Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) based on the Fenton Reaction. The Fenton reaction is the most common chemical reaction for the generation of hydroxyl radicals, which is proportional to the amount of superoxide anion. Typically, the Griess reagent turns red when an electron acceptor is given. There is a proportional relationship between the depth of color and the amount of hydroxyl radicals. The absorbance was detected using the scientific microplate reader (Thermo Multiskan Spectrum, Vantaa, Finland) at 510 nm.

Measurements of C-reactive protein (CRP) and plasma procalcitonin (PCT)

The level of serum CRP was determined by scatter rate nephelometry using BN-100 Automatic special protein analyzer (Dade Behring INC., USA). Meanwhile, the level of blood PCT was determined by immune chemiluminescence using quantification kit (Brahms Company, Berlin, Germany) according to the manufacturer's instructions.
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Table 1. The levels of endothelium injury markers in each group

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>VCAM-1 (ng/mL)</th>
<th>ICAM-1 (ng/mL)</th>
<th>Ang-2 (ng/mL)</th>
<th>TM (ng/mL)</th>
<th>AT-III (%)</th>
<th>TF (pg/mL)</th>
<th>VWF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>125.9±25.2</td>
<td>140.3±30.8</td>
<td>3.98±0.68</td>
<td>3.36±0.48</td>
<td>41.30±4.58</td>
<td>40.14±3.73</td>
<td>58.73±5.96</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>328.2±76.7</td>
<td>460.3±61.4</td>
<td>13.28±1.44</td>
<td>9.94±1.33</td>
<td>98.12±15.60</td>
<td>264.73±48.27</td>
<td>210.95±48.72</td>
</tr>
<tr>
<td></td>
<td>ROSI</td>
<td>168.6±38.4</td>
<td>195.7±38.1</td>
<td>4.59±0.72</td>
<td>4.16±0.82</td>
<td>52.67±10.34</td>
<td>84.83±10.12</td>
<td>85.79±10.12</td>
</tr>
<tr>
<td></td>
<td>GW9662</td>
<td>293.6±68.3</td>
<td>396.7±69.2</td>
<td>11.89±1.26</td>
<td>8.71±1.15</td>
<td>85.78±9.26</td>
<td>202.36±38.15</td>
<td>187.45±30.62</td>
</tr>
</tbody>
</table>

Note: VCAM-1: vascular cell adhesion molecule; ICAM-1: intercellular adhesion molecule; Ang-2: angiogenesis; TM: thrombomodulin; AT-III: antithrombin-III; TF: tissue factor; vWF: von willebrand factor. *P < 0.01 means the comparison with Control Group; #P < 0.01 and ##P > 0.05 mean the comparison with LPS Group.

Table 2. Variation of circulating endothelial cells (CEC) in each group

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>Control</th>
<th>LPS</th>
<th>ROSI</th>
<th>GW9662</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEC (cell/μL)</td>
<td>1.61±0.08</td>
<td>5.68±0.59</td>
<td>2.05±1.16</td>
<td>5.23±0.78</td>
<td></td>
</tr>
</tbody>
</table>

Note: *P < 0.01 means the comparison with Control Group; #P < 0.01 and ##P > 0.05 mean the comparison with LPS Group.

Table 3. The levels of oxidative stress-related factors in each group

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>NO (umol/L)</th>
<th>eNOS (U/L)</th>
<th>ROS (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>33.69±6.45</td>
<td>24.18±3.71</td>
<td>4.72±0.56</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>100.84±9.57</td>
<td>61.45±6.92</td>
<td>12.79±2.28</td>
</tr>
<tr>
<td></td>
<td>ROSI</td>
<td>45.18±6.59</td>
<td>31.75±4.16</td>
<td>6.83±1.26</td>
</tr>
<tr>
<td></td>
<td>GW9662</td>
<td>96.32±8.76</td>
<td>53.74±5.03</td>
<td>11.79±1.82</td>
</tr>
</tbody>
</table>

Note: NO: nitric oxide; eNOS: endothelial nitric oxide synthase; ROS: reactive oxygen species. *P < 0.01 vs control group; #P < 0.01 and ##P > 0.05 vs LPS group.

Results

PPAR-γ level increased by ROSI treatment and decreased by GW9662 treatment

In order to certify the effects of ROSI and GW9662 on the expression level of PPAR-γ, immunohistochemical analysis was conducted for rats in different groups (Figure 1). According to the results, no obvious difference exited between control group and LPS stimulation group. However, the expression level of PPAR-γ was remarkably increased in ROSI pretreatment group and notably decreased in GW9662 pretreatment group.

PPAR-γ agonist improved endothelium injury

The markers, including VCAM-1, ICAM-1, Ang-2, TM, AT-III, TF and vWF, were determined to evaluate the degree of endothelium injury (Table 1). After 4 hours of intravenous injection with LPS, all the levels of VCAM-1, ICAM-1, Ang-2, TM, AT-III, TF and vWF were significantly increased in LPS stimulation group compared with those in control group (P < 0.01). Meanwhile, the levels of all these markers in ROSI pretreatment group were significantly decreased compared with LPS Group (P < 0.01). However, no significant decrease was found in the levels of all these markers between GW9662 pretreatment group and LPS Group (P > 0.05).

PPAR-γ agonist significantly decrease the amount of CECs

Compared with control group, the amount of CECs was significantly increased after 4 hours of intravenous injection with LPS (Table 2, P < 0.01).
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Table 4. The levels of inflammation-related factors in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>TGF-β (pg/mL)</th>
<th>MMP-9 (ng/mL)</th>
<th>CRP (mg/L)</th>
<th>PCT (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.7±9.32</td>
<td>43.9±5.52</td>
<td>32.1±2.26</td>
<td>26.7±4.14</td>
<td>24.1±3.86</td>
<td>2.17±0.54</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td>LPS</td>
<td>268.6±42.74</td>
<td>389.0±68.47</td>
<td>67.8±8.64</td>
<td>58.9±7.48</td>
<td>67.5±5.99</td>
<td>89.7±14.56</td>
<td>67.3±14.77</td>
</tr>
<tr>
<td>ROSI</td>
<td>125.7±13.92</td>
<td>98.5±12.87</td>
<td>41.9±6.78</td>
<td>30.6±4.39</td>
<td>28.6±4.27</td>
<td>4.5±0.72</td>
<td>1.0±0.28</td>
</tr>
<tr>
<td>GW9662</td>
<td>220.7±39.29</td>
<td>379.4±59.74</td>
<td>59.7±7.96</td>
<td>49.0±6.05</td>
<td>61.3±5.01</td>
<td>81.4±14.02</td>
<td>60.2±10.11</td>
</tr>
</tbody>
</table>

Note: TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; IL-10: interleukin-10; TGF-β: transforming growth factor-β; MMP-9: matrix metalloprotein-9; CRP: C-reactive protein; PCT: plasma procalcitonin. *P < 0.01 vs control group; **P < 0.01; ***P < 0.001 vs LPS group.

0.01). Moreover, the amount of CECs in ROSI pretreatment group was significantly decreased compared with that in LPS group (P < 0.01), whereas it was without significant difference in GW9662 pretreatment group compared with LPS Group (P > 0.05) (Table 2).

The effects of PPAR-γ agonist on the enzyme activities of antioxidant system

As shown in Table 3, the activities of NO, eNOS and SOD were significantly decreased after 4 hours of intravenous injection with LPS as compared with those in the control group (P < 0.01). Meanwhile, the activities of these proteins were restored to high levels by ROSI pretreatment (P < 0.01). However, the effects of GW9662 pretreatment on the activities of NO, eNOS and SOD were similar and without statistical difference with those in LPS group (P > 0.05).

Changes in inflammation-related proteins expression

The levels of inflammation-related factors, TNF-α, IL-6, IL-10, TGF-β, MMP-9, CRP and PCT, were detected to assess the effects of PPAR-γ agonist on inflammation in Septic rats (Table 4). Compared with the control group, LPS treatment successfully caused severe inflammation with the notably higher levels of TNF-α, IL-6, IL-10, TGF-β, MMP-9, CRP and PCT (P < 0.01). As expected, the inflammation was significantly weakened by reducing these inflammation-related factors in ROSI pretreatment group (P < 0.01) whereas not in the GW9662 pretreatment group (P > 0.05).

Discussion

As the key points of sepsis, the endothelium was a major target of sepsis-induced events and endothelial damage was the main pathology leading to septic shock [19]. Various markers of endothelial activation and damage were increased during sepsis and systemic inflammation, which was correlated well with systemic inflammation, sepsis severity and outcome [20]. According to our results, PPAR-γ agonist (ROSI) improved endothelium injury by reducing oxidative stress and inflammation in septic rats.

ICAM-1 and VCAM-1 were members of the immunoglobulin family and adhesion molecules, which mediated firm adhesion between leukocytes and endothelium and subsequent diapedesis [21]. Widespread up-regulation of these adhesion molecules might cause tissue injury and multiple organ failure (MOF) during sepsis by facilitating recruitment and activation of leukocytes [21]. Thus, ICAM-1 and VCAM-1 might be good markers, indicating the sepsis-associated endothelium injury and inflammation. Ang-2 was an endothelial-specific growth factor which destabilized vascular endothelium and increased vascular leakage, thus might contribute to the pathophysiology of sepsis [22]. Orfanos et al. also found that patients with severe sepsis had elevated Ang-2 level of blood circulation and was positively correlated with inflammatory response [23]. The high vascular permeability and vascular leakage caused by excessively rising Ang-2 in blood circulation of patients with sepsis might be an important pathophysiology of sepsis. Moreover, Ang-2 levels were increased during severe sepsis and associated with disease severity and mortality [24]. Therefore, Ang-2 was expected to be the indicators which could reflect sepsis-associated endothelium injury and vascular leakage. In addition, coagulation might play an important role in the development of MOF in septic patients with critically ill [25]. TM expressed by endothelial cells acted on the anticoagulant side, while TF and vWF synthesized by endothelial cells acted on the procoagulant side [26]. TF-mediated pathways could lead to microvascular thromboses and endothelial activation dur-
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The endothelium injury related markers, including VCAM-1, ICAM-1, Ang-2, TM, TF and vWF, were greatly higher in LPS stimulation group than those in control group while significantly decreased in ROSI pretreatment group, suggesting that PPAR-γ agonist improved endothelium injury in septic rats. Consistently, the amount of CECs, a marker reflected the degree of vascular endothelial cells injury [28], was also significantly reduced in ROSI pretreatment group compared with LPS stimulation group. Thus, our results showed that PPAR-γ agonist might play an important role in inhibiting the injury of endothelial cells during sepsis.

Furthermore, the underlying mechanism for the effect of PPAR-γ agonist on endothelial injury was also exploited. Oxidative stress occurs in endothelial cells when imbalance existed between endogenous oxidative and antioxidant enzymes, which was contribute to the development of vascular disease [29]. Endothelial NO produced by eNOS played a major role in the homeostasis of normal endothelial cellular by regulating vascular tone and reducing proliferative state [29]. Vascular endothelium injury or dysfunction led to a decrease of NO production and altered blood vascular function [30]. Meanwhile, reduced expression, activity and/or activation of eNOS could reduce NO bioavailability [31]. A reducing oxidative stress could improve the endothelial function by regulating the eNOS activity and bioactive NO [32]. It had also been reported that enhancement of eNOS activity could decreased the interactions of neutrophil-endothelial cell induced by sepsis, which contributed to maintain the microvascular patency in septic shock [33]. Meanwhile, ROS in excess can trigger endothelium injury and excessive production of ROS had been considered as an important factor for the organ failure in sepsis pathogenesis [34]. The excess of ROS during sepsis could oxidize tetrahydrobiopterin which was a cofactor for eNOS [35]. According to our results, the levels of NO, eNOS and ROS were all significantly decreased in septic rats treated by ROSI, a PPAR-γ agonist, which indicated that PPAR-γ agonist might inhibit the endothelial cells injury by regulating the levels of NO, eNOS and ROS to decrease the oxidative stress.

PCT levels had been identified to be correlated with the severity of septic at onset, and PCT as well as CRP levels predicted a poor outcome of sepsis with a lower survival rate [36]. In this present study, the levels of PCT and CRP were increased in septic rats and then effectively suppressed by ROSI treatment. It had also reported that high PCT concentration was correlated with the severity of inflammation which had a predictive value for severe sepsis [37]. CRP was considered as an extremely sensitive indicator for sepsis diagnosis and it was also significantly elevated in inflammation process [38]. Additionally, PPAR-γ agonist ROSI had been shown to prevent leukocyte adhesion and reduce acute inflammation [39, 40]. Consistently, according to our study, ROSI inhibited the inflammation by significantly decreasing the levels of TNF-α, IL-6, IL-10, TGF-β and MPP-9 in septic rats. TGF-β levels were elevated in patients with sepsis [41] and the TNF-α and IL-6 level could provide valuable information regarding systemic inflammatory response in the diagnosis of sepsis [42]. Thus, we speculated that the inhibition effects of PPAR-γ agonist on inflammation might be benefit for the endothelium function recovery.

Conclusion

In conclusion, septic rat model inducted by LPS with severe endothelial damage was successfully established. PPAR-γ agonist could improve endothelial functions and reduce endothelial injury by suppressing oxidative stress and inflammation in septic rats. Therefore, PPAR-γ agonists may provide a broad prospect in the prevention and treatment of endothelial injury in sepsis. However, future studies should be performed to verify these investigations and clinical trials are needed.

Acknowledgements

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Disclosure of conflict of interest

None.

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