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Cardio protective Effects of Eritoran during Polymicrobial Sepsis through Decreases of p38MAPK/NF-κB Signaling Pathway

Nasser Ghaly Yousif[1]*, Najah R Hadi[2], Qassim A Zigam[2] and Ahmed N Altimimi[3]

[1]Department of Medicine, College of Medicine, Al Muthanna University, Iraq
[2]Department of Pharmacology and Therapeutics, College of Medicine, Kufa University, Iraq
[3]Ministry of Health, Iraq

Abstract

Background: Sepsis is a systemic inflammatory response usually correlates with multiorgan dysfunction. Myocardial dysfunction is one of adverse outcomes in septic patients resulted in high mortality rate.

Aim: To study the impact of eritoran in attenuation of cardiac depression during polymicrobial sepsis via decreased of phospho-p38MAPK/NF-κB signaling pathway.

Methods and Materials: Polymicrobial sepsis induced via cecal ligation and puncture model (CLP), in 8-12 weeks' age albino mice, 1 hr. prior to CLP mice were treated with IP Eritrean (5mg/kg). Twenty-four hours post CLP hemodynamic parameters including: heart rate, ejection fraction, LVEDP, LVSP and cardiac output, were carried out using micro-tipped transducer catheter. Plasma levels of proinflammatory cytokines, including TNF-α, IL-1β and IL-6, chemokine MCP-1 and cTn-I were measured via ELISA analysis. Phosphorylation degree of P38 MAPK and NF-κB carried out through western blot technique.

Results: Mice were treated with eritoran displayed improvement of LV function (Ejection fraction: 42.4 ± 1.1% versus 27.8 ± 3% in CLP mice). The attenuation of cardiac depression in eritoran treated mice was associated with lower levels of monocyte chemoattractant protein-1 (MCP-1) in plasma, and reduction in the levels of tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6). Furthermore, eritoran treated mice displayed less expression levels of phosphorylation p38-MAPK and NF-κB.

Conclusion: Eritoran can attenuate the cardiac dysfunction during polymicrobial sepsis possibly via a reduction of proinflammatory cytokines through decreased of both p38MAPK and NF-κB activation.

Correspondence to: Nasser Ghaly Yousif, Department of Medicine, College of Medicine, Al Muthanna University, Iraq

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Introduction

Sepsis is a systemic inflammatory reaction results from bacterial infection and considers as the master cause of death in critically ill patients [1,2]. Myocardial dysfunction is one of the major signs of adverse outcomes in septic patients, it usually correlates with decreased cardiac contractility, diastolic impairment and cardiac injury, provoking hypotensive condition, and approximately 25% of patients with sepsis have cardiovascular complications with elevated mortality rates up to 70% [2-4]. Polymicrobial sepsis may decrease cardiac work via rise expression level of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, which act as cardiodepressant proinflammatory mediators [5], resulting in cardiac contractile dysfunction [6], cardiac hypertrophy and heart failure [7,8]. Furthermore, increased cTn-I level during polymicrobial sepsis will decrease myofilaments calcium responsiveness to a large extent and subsequently impairment of cardiac contractile function will occur [9-11]. We proposed that eritoran in attenuation of cardiac depression during polymicrobial sepsis via decreased of phospho-p38MAPK/NF-κB signaling pathway

Materials and Methods

Experimental Animals

A total of 32 adult male albino Swiss mice aged 8-12 weeks, with weight of 20-30g, were obtained from Animal house, the College of Science, Babylon University. They were housed in the animal house of College of science, Kufa University. They were kept in cages under 12h light: 12h dark cycle, room temperature was kept at 25°C and humidity at 60-65%, with free access for food and water.

Study Design

Mice were assigned randomly to one of the following experimental groups (n = 8 in each group), Control group (CLP), Sham group (negative control), eritoran pretreated group (5 mg/kg of Eritoran 1hr prior to CLP), and Vehicle (PBS) pretreated group.

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CLP Procedure

In the present study, mice were selected to induce polymicrobial sepsis model based on previous studies [12-14]. In briefly, polymicrobial sepsis was induced by double puncture technique using 20 gage needles. Mice were anesthetized using ketamine/xylazine solution [15]. Laparotomy was done in abdomen via a 1.5 cm midline incision, the cecum was exposed. The cecum was ligated just below the ileocecal valve and punctured, and then the cecum was placed back in its anatomical position. The abdomen then sutured, using a 5.0 surgical suture (Ethicon, Norderstedt, Germany), 1 ml of Ringer's solution was given for resuscitation S.C, Mice were monitored for various signs of sickness every 4 hrs. for 24 hrs. Sham surgical operated mice (anesthesia and laparotomy) served as the surgical control group.

Hemodynamic Measurements

We assessed cardiac functions as described [16-18]. Briefly, mice were anesthetized intraperitoneally with ketamine in a dose of (50 mg/kg) post CLP. Animals were laid supine on a heating blanket and body temperature was maintained at range 37°C ± 0.5°C. The external right carotid artery was exposed, and a micro-tipped transducer catheter (1.4F, Millar Instrument Inc.) was placed into the artery and then advanced into the LV. The other end of the catheter was connected to an electrostatic chart recorder (model ES 2000, Gould, Cleveland, USA) and Pressure-volume loops recorded to measure the maximum rate of change in ventricular pressure and ejection fraction by using the MPVS-400 system with the aid of P van software (Conductance Technologies, San Antonio, TX, and Millar, Houston, TX) was used to measure all data. Heart rates, LV end-diastolic pressure (LVEDP), LV systolic pressure (LVSP).

ELISA Analysis

Commercial ELISA kits (Bosterbio corp.) were utilized to quantify the plasma levels of MCP-1, TNF-α, IL-1β, IL-6 and cTn-I. Samples and standardswere prepared according to manufacturer's instructions. Absorbance of standards and samples were determined spectrophotometrically at 450 nm, by a microplate reader (Bio-Rad Laboratories, CA, USA). Obtained data were plotted against the linear portion of a standard curve [19].

Western Blot Analysis

Myocytes of cardiac tissue were harvested with ice-cold PBS and centrifuged at 13,000×g for 3 min at 4°C. Nuclear and cytosolic extracts were prepared using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Institute of Biotechnology, Jiangsu, and China) according to the manufacturer's instructions. Protein concentrations were measured using a bicinchoninic acid protein assay kit (Beyotime Co, China). Equal amounts of lysate (50 μg) were separated on 10% SDS-PAGE. Proteins were transferred onto immunoblot polyvinylidene difluoride membranes (Chemico International, Millipore, Billerica, MA), and the membranes were blocked with 5% BSA in Tris-buffered saline with 0.1% Tween (TBS-T) for 2 hrs and incubated overnight at 4°C with the following primary antibodies; MAPK (1:1000), phospho-p38 (1:1000), p38 (1:1000), rabbit anti-mouse NF-κB (1:1000; Santa Cruz Biotechnology), β-actin (1:2000; Santa Cruz Biotechnology). Blots were washed four times for 15 min each in TBS-T and incubated with horseradish peroxidase-labeled secondary goat anti-rabbit (1:2000; Santa Cruz Biotechnology) or rabbit anti-goat (1:2000; Santa Cruz Biotechnology) for 1 h. Blots were again washed four times for 15 min each in TBS-T. Finally, blots were developed using the enhanced Chemiluminescence (Polygene Co, China) method.

Statistical Analysis

Statistical analysis data was performed using Stat View software (Abacus Concepts, USA). Analysis of variance (ANOVA) with Fisher post-hoc test was used to investigate differences between mice, and data differences were confirmed using the Mann-Whitney U-test. Statistically the present data significance was defined as $P \leq 0.05$.

Results

Effect of Eritoran pretreatment on the LV Function after CLP

To investigate the effect of treatment with Eritoran on the sepsis induced myocardial dysfunction, LV function was assessed 24 hrs. after CLP. The results in table 1 show that both CLP and vehicle groups have significantly ($p < 0.05$) attenuated LV function by decreased the levels of ejection fraction, cardiac output and LVESP with increase in heart rate and LVEDP ascompared with sham group. Furthermore; Eritoran pretreated group improved LV function through increased the levels of ejection fraction, cardiac output and LVESP with reduced heart rate and LVEDP.

Table 1: Effect of Eritoran pretreatment on hemodynamic status of mice 24 hrs. after CLP.

Parameter/ Groups	Heart rate (bpm)	LVEDP (mmHg)	Ejection fraction (%)	LVESP (mmHg)	Cardiac Output (ml/min)
Sham	403±12	3.2± 0.12	65.1±2	124.1±1.3	5.5±1.2
sepsis	466±13*	7.8± 0.14*	31.8±3*	54.2±1.6*	3.2±2.5*
Vehicle	458±11*	7.9± 0.12*	30.8±1.2*	54.7±1.4*	3.1±1.3*
Eritoran	432±15**	3.9± 0.12 **	54.3±2.4**	100.7±1.5**	4.9±2.6**

Data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; **P <0.05 *vs.* untreated.

Effect of Eritrean pretreatment on the plasma level of proinflammatory cytokines after CLP.

At the end of the experiment, (24 hrs. after CLP), the levels of plasma proinflammatory cytokines (TNF-α, IL-1β, and IL-6) measured by ELISA according to manufacture protocol the resulted data showed that all proinflammatory cytokines were significantly decrease in Eritoran pretreated group as compared with CLP and vehicle groups. The changes in plasma pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) levels are summarized in figure 1.

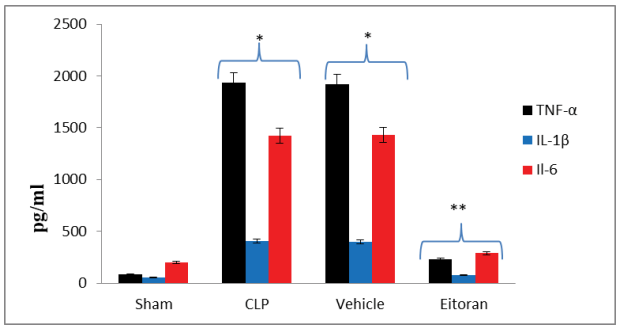

Figure 1: The mean of plasma proinflammatory cytokines (pg/ml) in the four experimental groups 24 hrs. after CLP. Data are expressed as mean ± standard error; *P <0.05 versus corresponding sham; **P <0.05 versus CLP mice.

Effect of Eritoran Pretreatment on the Plasma Level of MCP-1 after CLP

At the end of the experiment (24 hrs. after CLP), the level of plasma MCP-1 level measured by ELISA according to manufacturer protocol,

the resulted data showed that the plasma MCP-1 level was significantly decrease in Eritoran pretreated group as compared with CLP and vehicle groups. The changes in plasma MCP-1 level are summarized in figure 2.

Effect of Eritrean Pretreatment on Myocardial Injury after CLP

The plasma level of cTn-I was significantly ($p < 0.05$) increased in CLP and vehicle groups as compared with sham group. While, the cTn-I level of Eritoran pretreated group was significantly ($p < 0.05$) lower than that of CLP group. The changes in cTn-I level are summarized in figure 3.

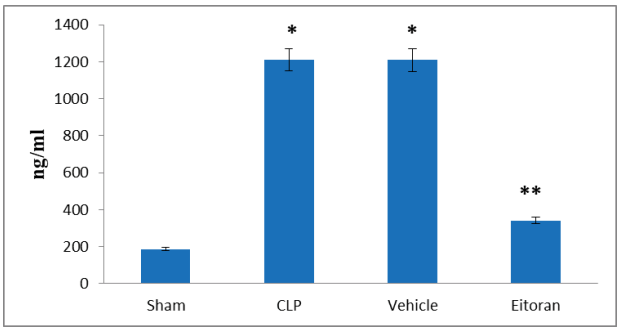

Figure 2: The mean of plasma MCP-1 level (ng/ml) in the four experimental groups 24 hrs. after CLP. Data are expressed as mean ± standard error; *P <0.05 versus corresponding sham; **P <0.05 versus CLP mice.

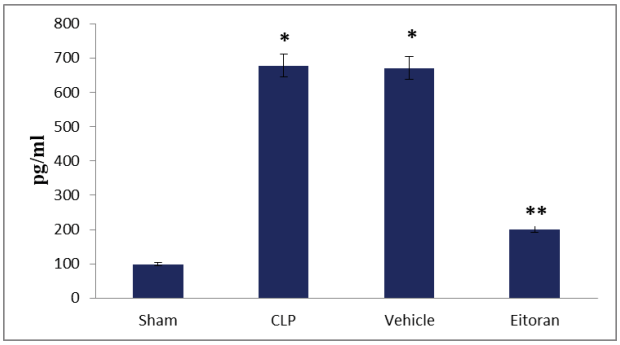

Figure 3: The mean of plasma cTn-I (pg/ml) in the four experimental groups 24 hour after CLP. Data are expressed as mean ± standard error; *P <0.05 versus corresponding sham; **P <0.05 versus CLP mice.

Eritoran Pretreatment Attenuates Phosphorylation of p38MAPK/NF-κB (Intracellular Signaling) in Cardiomyocytes after CLP

Myocardial tissue homogenates were analyzed using western blot technique. The p38MAPK/NF-κB phosphorylation in myocardial cells was significantly ($p < 0.05$) increased in CLP and vehicle groups as compared with sham group. While, the NF-κB phosphorylation in Eritoranpretreated groups was significantly ($p < 0.05$) lower than that of CLP group. This indicates theinvolvement of NF-κB in the mechanistic action of Eritoran. The phosphorylated p38MAPK level of Eritoranpretreated group was significantly ($p < 0.05$) lower than that of CLP group. This indicates the involvement of p38MAPK in the mechanistic action of Eritoran. The changes in p38MAPK and NF-κB level are summarized in figure 4.

Discussion

During sepsis, the inflammatory responses mediate myocardial injury, including LV dysfunction and cardiac pathophysiological

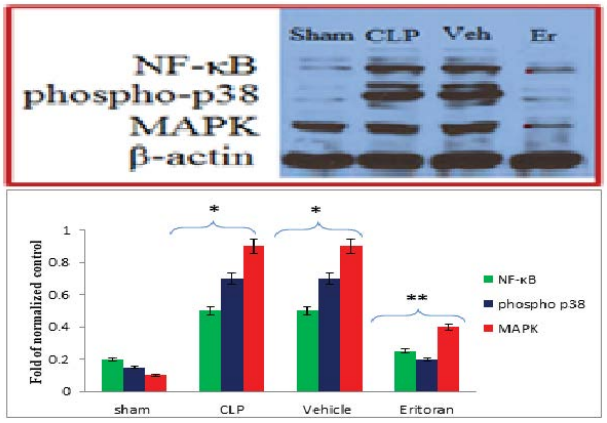

Figure 4: The mean of relative p38MAPK/NF-κB activity in the four experimental groups 24hr after CLP. Data are expressed as mean ± standard error; *P <0.05 versus corresponding sham; **P <0.05 versus CLP mice.

changes [20-22]. Previous studies reported that plasma levels of inflammatory mediators (IL-1β, TNF-α, and IL-6) were higher following myocardial injury and sepsis [23-25]. It was also found that in vivo sepsis mice model and LPS-mediated increased MCP- 1 levels in both plasma and myocardial tissue [26]. To understand the pathway of sepsis related in the vulnerability to end toxemic cardiac depression, the present study investigated the role of Eritoran pretreatment in improving the cardiac function following sepsis and possible pathway. According to our knowledge there was no data published discussed the relationship between p38MAPK/NF-κB pathway and effective role of Eritoran on improved cardiac function following sepsis by CLP model in mice.

Sepsis Attenuated Myocardial Function through Elevation of Inflammatory Mediators

A number of published paper have investigated and confirmed that myocardial dysfunction during sepsis is related with inflammatory mediator's expression, including IL-6, TNF-α, and IL- 1β [26,27]. Furthermore, inflammatory cytokines have been upregulated in myocardial dysfunction in clinical aspects after acute injuries caused by sepsis, myocardial ischemia and reperfusion [26-29]. Additionally, intravenous administration of either TNF-α or IL-1β in animal experiments can evoke a similar process to that caused by sepsis lead to cardiac comorbidity and mortality [26], and this adverse effects of pro-inflammatory cytokines can be ameliorated by antibodies like that antagonize the effects of these molecules [29-31]. Other studies demonstrated that TNF-α also plays an important role in the septic myocardial dysfunction and that TNF-α links TLR4 activation pathway [30, 32-34]. In the present study, we demonstrated that sepsis increases the levels of inflammatory mediators (IL-1β, TNF-α and IL-6) in both plasma and cardiac tissue of mice, that associated with worse LV function performance through the hemodynamic measurements (heart rate, ejection fraction) and these results are associated with increased the levels of circulating cTn-I in mice exposed to CLP. Our data suggest that significantly higher levels of myocardial depressant pro-inflammatory cytokines in the heart directly attenuated cardiac contractility and induce myocardial injury together of these results contribute, in some part, to the mechanism of exaggerated cardiac depression in experimental sepsis mice model. Interestingly, we observed that pretreatment with Eritoran resulted in a greater reduction in cytokines with improvement in LV function, ejection fraction was improved in Eritoranpretreated mice. Additionally, pretreatment with

Eritoran improves otherLV function parameters, such as LVESP and cardiac output.

Sepsis Up-Regulated Myocardial MCP-1 Expression Level

Many studies demonstrated that antagonized of MCP-1 has been shown to decreased neutrophils recruitment and reduced tissue injury in many animal models of sepsis-induced organs injury [35-37]. In the present data, we investigated that plasma level of MCP-1 was significantly higher in the CLP than sham mice.

Down-Regulation of p38MAPK/NF-κB Improved LV Function

The intracellular downstream signaling pathway of TLR4 includes phosphorylation of p38MAPK and activation of NF-κB [10,11, and 38-40]. In the present work, we investigated the action of Eritoran in mechanistic view, which suggested that Eritoran decreased the cardiac injury in Eritoran pretreated group through its ability to decrease both the degree of p38MAPK phosphorylation and the NF-κB activation as compared with CLP group that sow elevated level of p38MAPK phosphorylation and the NF-κB activation.

Conclusion

This study found that both p38MAPK phosphorylation and NF-κB activation are increased during sepsis and lead to attenuation of LV function. Additionally, it was found that both p38MAPK phosphorylation and NF-κB activation is closely related to increase the plasma level of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, which lead to further decreased in LV function, that lead as to suggest that both p38MAPK phosphorylation and NF-κB activation could be a biomarkers and novel target for therapy in patients with cardiac complications during sepsis via improvement of LV function. These experimental results let us suggest that endogenous p38MAPK phosphorylation and NF-κB activation mediates the expression of MCP-1, led to increased level cTn-I with sequential signal caused myocardial cell injury. The western blot really showed that there were low levels of both p38MAPK and NF-κB in Eritoran pretreated mice compared with untreated or vehicle mice. The effects of p38 MAPKor NF-κB inhibitors during sepsis remain to be further studied and tested.

Conflict of Interest

There is no conflict of interest regarding the publication of this paper.

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