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Research Article

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The Cardio Protective Potential of Irbesartan during Polymicrobial Sepsis through Modulation of P38mapk/NF-Kb Signaling Pathway

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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 irbesartan on TLR4 in attenuation of cardiac depression during polymicrobial sepsis via modulation of p38MAPK/NF-κB signaling.
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 irbesartan (3mg/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: Hemodynamic parameters showing that irbesartan pretreated group had significantly ($p<0.05$) elevated ejection fraction, LVSP and cardiac output and significantly ($p<0.05$) decreased in heart rate and LVEDP as compared with vehicle and CLP groups. Proinflammatory cytokines (TNF-α, IL-1β and IL-6), MCP-1 and cTn-I were significantly ($p<0.05$) lower in irbesartan pretreated group than vehicle and CLP groups. Western blot analysis shows that phosphorylation degree of p38 MAPK and NF-κB in irbesartan pretreated group were significantly ($p<0.05$) lower than vehicle and CLP groups.
Conclusion: Irbesartan can attenuate the cardiac dysfunction during polymicrobial sepsis possibly via a reduction of proinflammatory cytokines through modulation of both p38MAPK and NF-κB activation.

Keywords: Cardio Protective; Sepsis; Irbesartan

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, approximately one fourth of patients with sepsis have cardiovascular complications.with elevated mortality rates up to 70% [2-4]. Endotoxemia 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]. Furthermore, increased cTn-I level during endotoxemia will decrease myofilaments calcium responsiveness to a large extent and subsequently impairment of cardiac contractile function will occur [8].
TLR4, a member of the TLRs family, provokes immune responses by stimulation inflammatory cascades during sepsis through binding to bacterial lipopolysaccharide. Subsequently to its specific ligand binding, TLR4 and its

related co-receptors, cluster of differentiation 14 (CD14) and myeloid differentiation factor 2 (MD-2), recruit adaptor proteins that encourage downstream signaling via activation of NF-κB and p38MAPK [9-11].
We proposed that irbesartan can attenuate cardiac dysfunction during poly microbial sepsis through blockade of TLR4 signaling and modulates its downstream activation of transcription factors that involved in proinflammatory mediators production.

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 kept in cages under 12h light: 12h dark cycle, room temperature was kept at 25°C and humidity at 60–65%, with free excess 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), irbesartan pretreated group: (3mg/kg of irbesartan 1hr prior to CLP), and Vehicle (PBS) pretreated group.

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, 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 hours for 24 hours. 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

The samples of blood from mice were centrifuged (in 10000 RPM, for 10 minutes) and myocardial tissue was homogenized and treated in PBS containing 0.5% Triton X100 with a protease inhibitor cocktail. Commercial ELISA kits (Bosterbio corp.) were utilized to quantify MCP-1, TNF-α, IL-1β and IL-6 in plasma and myocardial tissue, and plasma cardiac Troponin-I (cTn-I). Samples and standards were 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

Cells 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, China) according to the manufacturer's instructions. Protein concentrations were measured using a bicinchoninic acid protein assay kit (Beyotime Co, China). Equal amounts of lysates (50 μg) were separated on 10% SDS-PAGE. Proteins were transferred onto immunoblot polyvinylidene difluoride membranes (Chemicon International, Millipore, Billerica, MA), and the membranes were blocked with 5% BSA in Tris-buffered saline with

0.1% Tween (TBS-T) for 2 h 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 (Pplygen Co, China) method.

RESULTS

Effect of irbesartan pretreatment on the LV Function after CLP

To investigate the effect of treatment with irbesartan on the sepsis induced myocardial dysfunction, LV function was assessed 24 hours 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 as compared with sham group. Further irbesaran treated 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 irbesartan pretreatment on hemodynamic status of mice 24 hours after CLP

Parameters\Groups	Sham	CLP	Vehicle	Irbesartan
HR (bpm)	403 ± 12	466 ± 13*	458 ± 11*	461 ± 2**#
LVEDP (mmHg)	3.2±1.2	7.8±1.4*	7.9±1.2*	4.8±1.3**#
Ejection fraction (%)	65.1 ± 2	31.8 ± 3*	30.8 ± 1.2*	40.4 ± 1.1**#
LVESP (mmHg)	124.1±1.3	54.2±1.6*	54.7±1.4*	82.5±3.2**#
Cardiac Output (ml/min)	5.5 ± 1.2	3.2 ± 2.5*	3.1 ± 1.3*	3.4 ± 1.9**#

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

Effect of irbesartan pretreatment on the plasma level of proinflammatory cytokines after CLP

At the end of the experiment, (24 hour 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 increased after CLP and vehicle treated compared with irbesartan treated group. The changes in plasma pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) levels are summarized in figure (1).

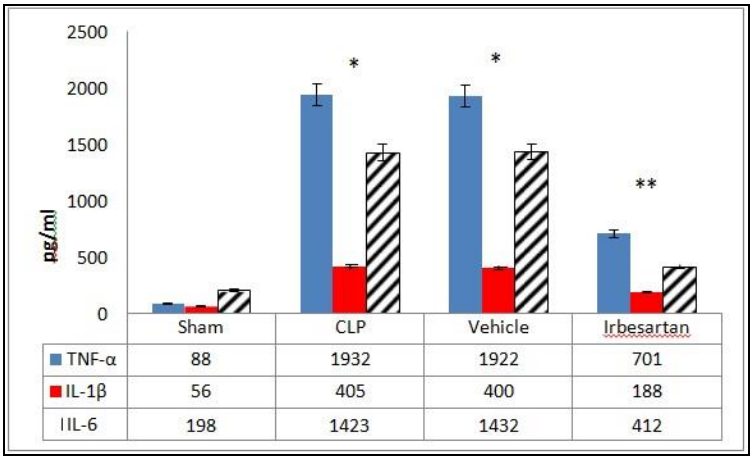

Figure 1: The mean of plasma proinflammatory cytokines (pg/ml) in the four experimental groups 24 hour after CLP

Data are expressed as mean ± standard error; * P <0.05 versus corresponding sham; ** P <0.05 versus CLP mice.

Effect of irbesartan pretreatment on the plasma level of MCP-1 after CLP

At the end of the experiment (24 hour 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 increased after CLP and vehicle treated compared with irbesartan treated group. The changes in plasma MCP-1 level are summarized in figure (2).

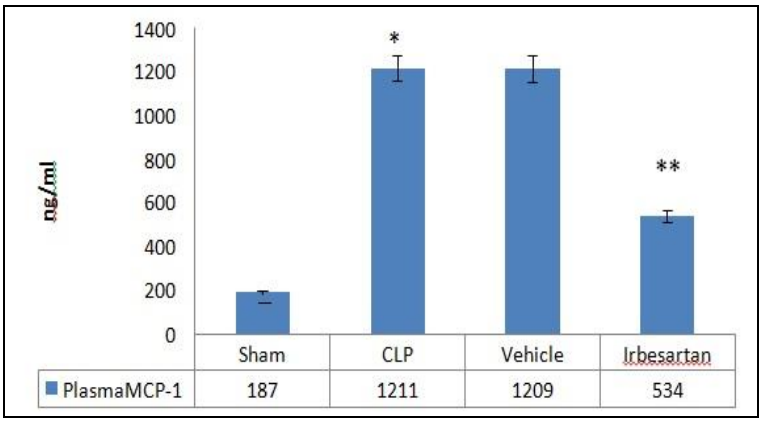

Figure 2: The mean of plasma MCP-1 level (ng/ml) in the four experimental groups 24 hour after CLP

Data are expressed as mean ± standard error; * P <0.05 versus corresponding sham; ** P <0.05 versus CLP mice.

Effect of irbesartan 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. The cTn-I level of irbesartan treated group was significantly ($p < 0.05$) lower than that of CLP group. The changes in cTn-I level are summarized in figure (3).

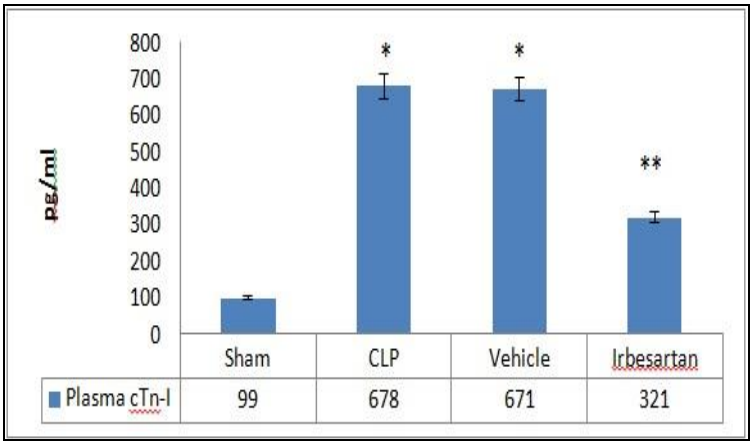

Figure 3: The mean of plasma cTn-I (pg/ml) in the four experimental groups 24 hour after CLP

Data are expressed as mean ± standard error; * P <0.05 versus corresponding sham; ** P <0.05 versus CLP mice.

Irbesartan pretreatment attenuates phosphorylation of p38MAPK/NF-κB (intracellular signaling) in cardiomyocytes after CLP

Myocardial tissue homogenates were analyzed using western blot technique. The p38 MAPK / NF-κB phosphorylation in myocardial cells was significantly ($p < 0.05$) increased in CLP and vehicle groups as compared with sham group. The NF-κB phosphorylation in irbesartan treated groups was significantly ($p < 0.05$) lower than that of CLP group. This indicates the involvement of NF-κB in the mechanistic action of irbesartan. The phosphorylated p38MAPK level of irbesatan treated group was significantly ($p < 0.05$) lower than that of CLP group. This indicates the involvement of p38MAPK in the mechanistic action of irbesartan. The changes in p38MAPK and NF-κB level are summarized in figure (4).

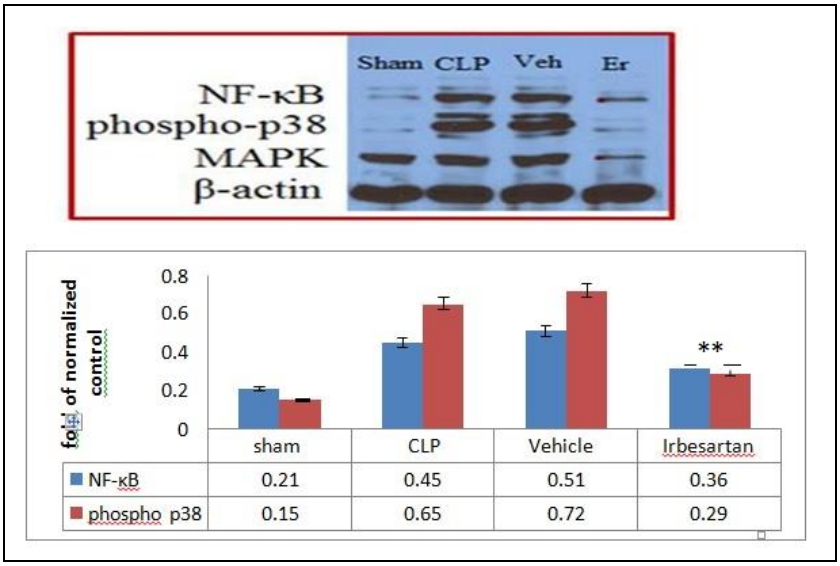

Figure 4: The mean of relative p38MAPK/NF-κB activity in the four experimental groups 24 hour after CLP

Data are expressed as mean ± standard error; * P <0.05 versus corresponding sham; ** P <0.05 versus CLP mice.

DISCUSSION

During sepsis, the inflammatory responses mediate myocardial injury, including LV dysfunction and cardiac pathophysiological 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 endotoxemic cardiac depression, the present study investigated the role of irbesartan 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 irbesaran 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, 28, 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]. 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 pre-treatment with irbesartan resulted in a greater reduction in cytokines with improvement in LV function, ejection fraction was improved to (40.4 ± 1.1) in irbesartan treated mice. Additionally, pretreatment with irbesartan improves other LV function parameters, such as LVESP (82.5±3.2) and cardiac output (3.4 ± 1.9).

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[33-34]. In the present data, we

investigated that MCP-1 levels in plasma and cardiac tissue are significantly higher in the CLP than sham mice.

Down-regulation of p38MAPK/NF-κB improved LV function

The intracellular downstream signaling pathway of TLR4 include phosphorylation of p38MAPK and activation of NF-κB [10,11,35,36]. In the present work, we investigated the action of irbesartan in mechanistic view, which suggested that irbesartan decreased the cardiac injury in irbesartan 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 and tissue level elevation 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 irbesartan pretreated mice compared with no treated or vehicle mice. The effects of p38 MAPK or NF-κB inhibitors during sepsis remain to be further studied and tested.

Statistical analysis

Statistical analysis data was performed using StatView 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 $P \leq 0.05$.

Conflict of interest: There is no conflict of interest regarding the publication of this paper

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