

The Cardioprotective Effects of Atorvastatin in Experimental Endotoxemia

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Abstract

Sepsis, a potentially fatal illness caused by infection, frequently leads to the failure of multiple organs, including the heart. This study aimed to investigate the potential mechanism by which atorvastatin improves heart function following sepsis. A total of 35 male Swiss albino mice, weighing between 30 and 38 grams and aged 8 to 12 weeks, were randomly allocated into five groups. Each group consisted of seven animals. The control group maintained their regular diet until the designated sampling time. The sham group underwent laparotomy and anesthesia. The sepsis group underwent the cecal ligation and puncture procedure. The vehicle group received intraperitoneal injections of an equivalent volume of dimethyl sulfoxide (DMSO) for five days, followed by the cecal ligation and puncture procedure. The Atorvastatin group received intraperitoneal injections of 20 mg/kg of Atorvastatin for five days after the cecal ligation and puncture procedure. Twenty hours following the cecal ligation and puncture procedure, the mice were humanely killed and samples of serum and heart tissue were obtained. The serum levels of NF-KB, FOXO3A, CASPASE3, and NT-PROPNB were evaluated. The data, which followed a normal distribution, was examined using t-tests and ANOVA tests with a significance level of $p < 0.05$. The group of sepsis exhibited considerably elevated levels of NF-KB, FOXO3A, CASPASE3, and NT-PROPNB compared to the sham group. However, the pre-treated group with Atorvastatin demonstrated significantly reduced levels ($p < 0.05$) of these markers compared to the sepsis group. The histological characteristics of mice treated with Atorvastatin showed slight variations in comparison to control and sham groups. Our inquiry findings suggest that Atorvastatin exhibits anti-apoptotic and cardioprotective properties in cases of polymicrobial sepsis; this is supported by a significant reduction in levels of NF-KB, FOXO3A, CASPASE3, and NT-PROPNB in the bloodstream.

KEYWORD: sepsis, Atorvastatin, CLP, NF-KB, FOXO3A, CASPASE3, and NT-PROPNB.

Introduction

Sepsis is a serious condition that results from an uncontrolled body response to an infection, resulting in a severe malfunction of the organs 1. Epidemiological studies have identified a global population of around 18 million individuals who are diagnosed with sepsis annually, and the mortality rate for this condition ranges from 28% to 40%. The fatality rate of those suffering from septic shock is significantly higher. Septic cardiomyopathy is a reversible impairment of the heart muscle that arises as a result of sepsis-induced multi-organ failure. The characteristic clinical manifestations of early sepsis predominantly arise from an excessive and inflammatory immune response, prompting numerous medication trials aimed at inhibiting these pro-inflammatory effects 4. Sepsis typically begins when components of the innate immune system simultaneously detect either pathogen-

associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) 5 6. Apoptosis is crucial in the process of sepsis-induced immunosuppression as it helps in choosing immune cell populations and ensuring the proper functioning of immune responses 2. Apoptosis in the innate and adaptive immune systems has a positive impact by mitigating the inflammatory response; sepsis can be controlled in the host. Nevertheless, the substantial reduction in immune cells can compromise the host's capacity to efficiently eliminate invading infections 7. Apoptosis of immune cells in lymphoid tissues and gut-associated lymphoid tissues can lead to a significant reduction of immune cells, such as monocytes, macrophages, dendritic cells, natural killer cells, and B cells 8. An immune cell apoptosis brought on by sepsis may compromise host immunity by causing anemia, reactivating latent infections, and increasing vulnerability to secondary infections 9. Caspases, which are cysteine proteases, play a crucial role in apoptosis by breaking down cellular proteins. Another key component is the nuclear factor-kappa B (NF- κ B), a transcription factor that triggers the transcription of genes involved in both promoting cell death and cell survival. The hyper-inflammatory reactions of sepsis rely on NF- κ B to produce pro-inflammatory cytokines and activate caspases through cleavage. Both NF- κ B and caspases also simultaneously trigger the apoptosis of immune cells 10. Promising new treatment prospects arise from the capacity to progressively monitor uncontrolled lymphocyte death as a way to assess the effectiveness of immune-adjuvant therapy, given the substantial immunosuppression caused by immune cell depletion that occurs during sepsis 11.

AIM

This study aims to assess the cardioprotective effect of atorvastatin during polymicrobial sepsis in male mice.

Material and Methods

Animal Preparation

Thirty-five mature albino male Swiss mice were obtained from the Iraqi Center for Cancer Research. The mice weighed between (30 -38 g) were kept at The College of Pharmacy's Animal house at the University of Kufa. The mice were confined in enclosures that followed a 12-hour cycle of alternating light and darkness. The temperature in the enclosures ranged from 22 to 24 °C, while the humidity level ranged from 60 to 65%. The subjects had unlimited access to water and food. Institutional Animal Care approved the study and Use Committee (IACUC) of Kufa University, Research was conducted in the Laboratory of Clinical and Laboratory Department, college of Pharmacy, University of Kufa, spanning from December 5, 2023, to February 25, 2024.

Study design

The animals have been categorized into five groups, each consisting of seven animals, as shown below:

- Control group: The mice were provided with their regular diets until the time of sample collection.
- Sham group: Mice underwent laparotomy and anesthesia. The sham group represents the negative surgical control group.
- The Sepsis group: The mice underwent the cecal ligation and puncture (CLP) technique. The Sepsis group serves as the control group for surgical procedures with positive outcomes.
- The Vehicle group: The mice in this group were administered intraperitoneal injections of DMSO at a consistent amount for five consecutive days, after which they underwent CLP.

Experimental Model of Sepsis

Recent research has utilized mice to produce polymicrobial sepsis through the CLP model 12. The mice were administered intraperitoneal anesthesia using a mixture of 100 mg/kg of ketamine and 10 mg of Xylazine 13.

The abdomen of the mouse was shaved and disinfected. Then, a midline incision of 1.5 cm was made for abdominal laparotomy, which exposed the cecum. Subsequently, the cecum was securely tied immediately below the ileocecal valve, punctured twice with a G-22 needle, and softly compressed to extract a small amount of stool from the puncture before being returned to its original position. Subsequently, a 5/0 surgical suture was employed to seal the abdominal incision. By administering a subcutaneous injection of 1 ml of a 0.9% saline solution, the mice were revived, subsequently; the mice were monitored every 4 hours for a duration of 24 hours before being returned.

Atorvastatin preparation

Atorvastatin was obtained as pure powder from MedChem Express(MCE),CHINA. Atorvastatin was solubilized in dimethyl sulfoxide (DMSO). Subsequently, a dose of 20mg for each kg of atorvastatin was delivered intraperitoneally for a duration of five days, following the CLP procedure.

Samples Collection

Blood Samples

Blood samples were gathered by heart puncture before the mice were sacrificed. A gel tube was placed and kept for one hour at room temperature. Blood was centrifuged for 20 minutes at 4000 rpm to separate the serum. The amount of NT-PROBNP was quantified using the enzyme-linked immunosorbent assay (ELISA) method.

Tissue preparation for ELISA assessment of NF-KB, FOXO3A and CASPASE3

The cardiac tissues were washed with a solution of 0.9% sodium chloride to eliminate any traces of blood before being preserved at a temperature of -80 degrees Celsius in a deep freezer. The heart sections were homogenized using a mortar and pestle and then processed with a high-intensity ultrasonic liquid processor in a phosphate buffered saline solution containing 1% TRION X-100 and 1% protease inhibitor cocktail. The tissue homogenate was centrifuged at a speed of 3000 revolutions per minute for 20 minutes at a temperature of 4 degrees Celsius. Subsequently, levels of NF-KB, FOXO3A and CASPASE3 were measured in the supernatant of the tissue homogenate in accordance with the manufacturer's instructions for ELISA kits.

Tissue Preparation for Histopathology

The cardiac tissue collected after sacrificing the mouse was washed with a cold solution of isotonic sodium chloride (0.9 percent). The heart tissue was fixed in a 10% formaldehyde solution for a duration of 20 hours. Following the process of dehydration and cleaning, the heart tissue was placed in a paraffin block and then sliced into a five μm -thick segment using a microtome. Tissue samples were subjected to staining using the hematoxylin and eosin (H&E) procedure.

Histological Examination

Cardiac damage was evaluated for each segment of the heart using an optical microscope, and images of the sections were recorded. Utilizing Zingarelli's methodology, histological sections from all groups were evaluated and categorized to approximately ascertain the degree of difference in heart injury. The parameters for this evaluation method were:

- Score zero: there is no harm or destruction: (normal tissue).
- Score 1: a localized necrosis and interstitial edema (mild).
- Score 2: a Swelling of cardiac cells and widespread necrosis (moderate).
- Score: 3 an Ischemia with an accumulation of neutrophils: (severe).
- Score 4: contractile bands, bleeding, ischemia, and leukocyte infiltration (very severe).

Statistical Analysis

Graph Pad Prism version 8.1 was used to do the statistical analysis. The data was displayed using the mean \pm standard error mean (SEM). one-way analysis of variance (ANOVA) test was used in this investigation to assess the disparities between the experimental groups. Post hoc tests were then performed using Bonferroni several comparisons method. Dunn's post hoc testing and non-parametric tests were used to compare the histopathological alterations between the groups. In statistics, a test was deemed significant if the P value achieved was less than 0.05.

Results

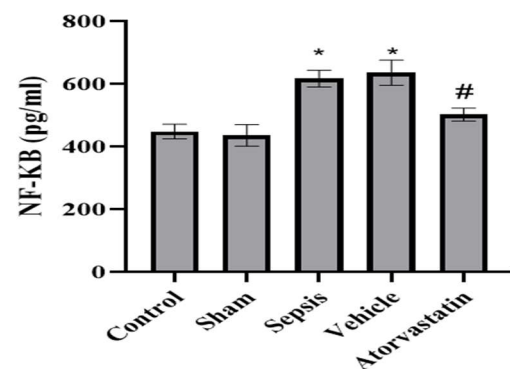
The Effect of atorvastatin Treatment on NF- κ B Level

The mice in the sepsis group exhibited markedly elevated blood NF- κ B levels compared to the mice in the sham and normal groups ($P < 0.05$). There was no statistically significant variation in serum NF- κ B levels reported between the vehicle and sepsis groups, as well as between the sham and normal groups. (Figure1). The atorvastatin treated group showed a notable lesson in blood NF- κ B levels as opposed to the sepsis and vehicle groups ($P < 0.05$).

Figure 1: Serum level of NF- κ B in experimental group

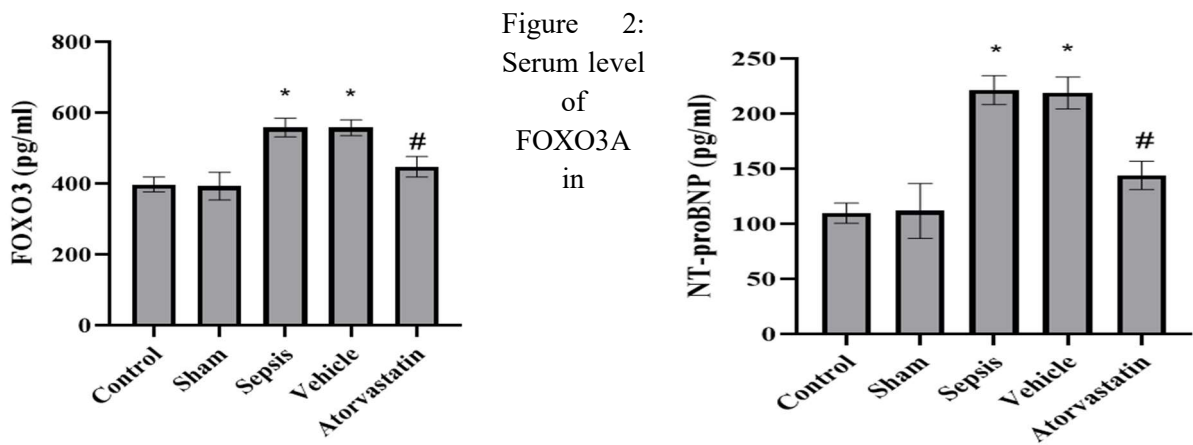
*: Significantly, distinct ($p < 0.05$) compared to the control or Sham group.

#: significantly, distinct ($p < 0.05$) compared to control or sepsis or vehicle group the



The Effect of atorvastatin Treatment on FOXO3A Level

The mice in the sepsis group exhibited markedly elevated blood FOXO3A levels compared to the mice in the sham and normal groups ($P < 0.05$). There was no statistically significant variation in serum FOXO3A levels reported between the vehicle and sepsis groups, as well as between the sham and normal groups. (Figure2). The group treated with atorvastatin showed a notable lesson in blood FOXO3A levels as opposed to the sepsis and vehicle groups ($P < 0.05$).



experimental groups

*: Significantly, distinct ($p < 0.05$) compared to the control or Sham group.

#: significantly, distinct ($p < 0.05$) compared to the control or sepsis or vehicle group.

The Effect of atorvastatin Treatment on CASPASE3 level

The mice in the sepsis group exhibited markedly elevated blood

CASPASE3 levels compared to the mice in the sham and normal groups ($P < 0.05$). There was no statistically significant variation in serum CASPASE3 levels reported between the vehicle and sepsis groups, as well as between the sham and normal groups. (Figure 3). The group treated with atorvastatin showed a notable lesson in blood CASPASE3 levels as opposed to the sepsis and vehicle groups ($P < 0.05$).

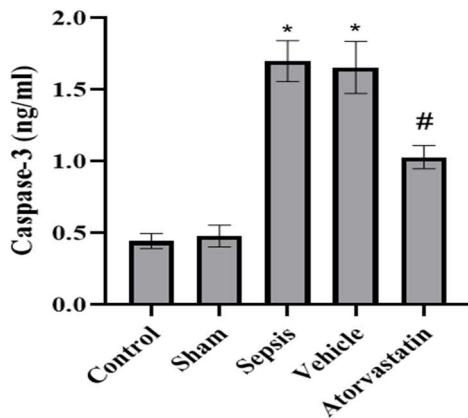
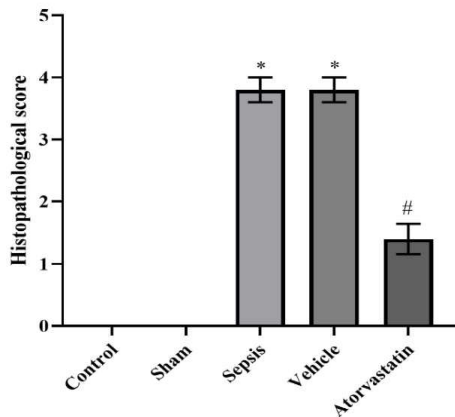


Fig3: Serum level of CASPASE3 in experimental groups.

*: Significantly, distinct ($p < 0.05$) compared to the control or Sham group.

#: significantly, distinct ($p < 0.05$) compared to the control or sepsis or vehicle group



3.6 The Effect of atorvastatin Treatment on NT-PROBNB Level

The mice in the sepsis group exhibited markedly elevated blood NT-PROBNB levels compared to the mice in the sham and normal groups ($P < 0.05$). (Table 3.6) There was no statistically significant variation in serum NT-PROBNB levels reported between the vehicle and sepsis groups, as well as between the sham and normal groups. (Figure 4). The atorvastatin treated group showed a notable lesson in blood NT-PROBNB levels as opposed to the sepsis and vehicle groups ($P < 0.05$).

Fig4: Serum level of NT-PROBNB in experimental groups.

*: Significantly, distinct ($p < 0.05$) compared to the control or Sham group.

#: significantly, distinct ($p < 0.05$) compared to the control or sepsis or vehicle group.

HISTOPATHOLOGICAL CHANGES OF MYOCARDIAL TISSUE AFTER POLYMICROBIAL SEPSIS

Myocardium tissue of the normal and sham groups showed the tissue of the heart had normal architecture, with defined borders between the myocytes and no erythrocyte leakage or leukocyte infiltration (fig. 5. A-B). Every mouse in both normal and sham group exhibits typical histopathology results (score 0) as shown in (Fig. 6).

The sepsis and vehicle groups both have sever myocardial damage (score 3) characterized by a severe combination of acute and chronic inflammatory cell infiltration, consisting of neutrophils and lymphocytes. Mild edema and significant congestion of blood vessels accompanied this infiltration. (Fig.5.C-D). Mean histopathological scores were considerably higher in vehicle and sepsis groups compared to normal and sham groups ($P < 0.05$), (fig.6).

Atorvastatin –treated group had mild myocardial injury (score 1) (fig. 5E). The average histopathological score was lower in the atorvastatin-treated group in contrast to the sepsis and vehicle groups ($P < 0.05$), (fig6).

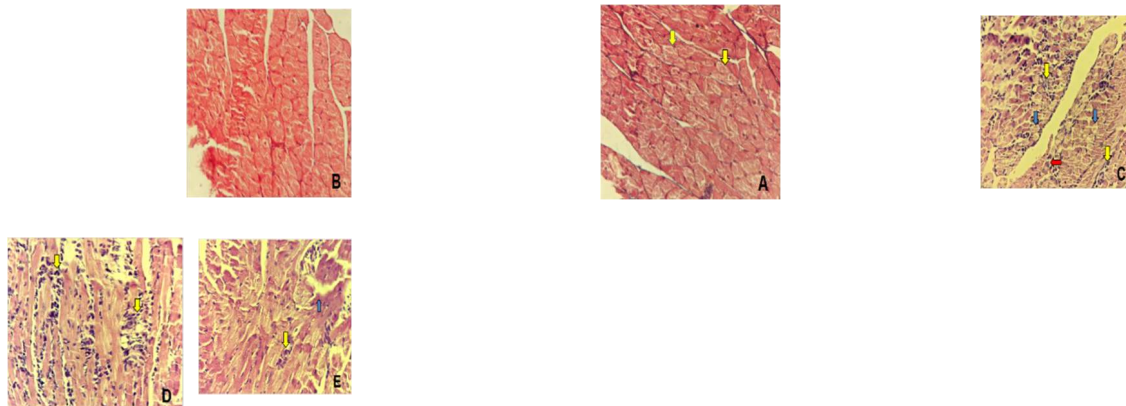


Figure 5: Photograph for cardiac section that stained with H&E (X400). A: normal group showed normal architecture (score 0); B: the sham group showed normal architecture (score 0); C: the sepsis group, showed

inflammation (yellow arrows), edema (blue arrows) and congestive blood vessel (red arrow); D: the vehicle group showed inflammation (yellow arrow); E: the Atorvastatin group showed mild inflammation (yellow arrows) and mild edema (blue arrow)

Figure 6: Histopathological scores in experimental groups. * Significant versus sham or normal groups ($P < 0.05$).

. # Significant versus sepsis or vehicle groups ($P < 0.05$).

DISCUSSION

Sepsis, a condition characterized by physiological, pathological, and metabolic abnormalities resulting from infection, is a significant global public health issue that requires substantial financial resources. It is the primary cause of mortality in critical care units 19. The nuclear transcription factor NF- κ B is crucial in initiating a cytokine storm by activating the expression of genes that promote inflammation. Several stimuli trigger NF- κ B, with the primary ones being bacterial pathogens detected by Toll-like receptor 4 (TLR-4) and pro-inflammatory cytokines recognized by particular membrane receptors. 20 .

Significantly higher serum levels of NF- κ B were found in the vehicle and sepsis groups compared to the normal and sham groups in this study. In contrast, the Atorvastatin -treated group had markedly lower NF- κ B serum levels than the vehicle and sepsis groups. 21 had provided the same results.

This study also discovered markedly elevated serum levels of FOXO3A in the vehicle and sepsis groups in comparison to the control and sham groups In contrast, the Atorvastatin -treated group had markedly lower FOXO3A serum levels than the vehicle and sepsis groups.

22 demonstrated that the transfer of FoxO3a into the nucleus and subsequent activation of atrophic markers MuRF-1 and MAFbx occur after treatment with IGF-1 and/or simvastatin. As far as we are aware, no more information regarding the impact of Atorvastatin on FOXO3A is presently accessible.

This study also discovered markedly elevated serum levels of caspase 3 in the vehicle and sepsis groups in comparison to the control and sham groups In contrast, the Atorvastatin -treated group had markedly lower caspase 3 serum levels than the vehicle and sepsis groups.

Pre-treatment with Atrovastatin resulted in the suppression of caspase 3 and TNF- α , which led to enhanced cardiac function and reduced levels of serum cardiac troponin I (cTn-I) and high-sensitivity C-reactive protein 23.

In addition this study found that markedly elevated serum levels of NT-proBNP in the vehicle and sepsis groups in comparison to the control and sham groups In contrast, the Atorvastatin -treated group had markedly lower NT-proBNP serum levels than the vehicle and sepsis groups.

24 demonstrated that administration of atorvastatin resulted in a decrease in NT-proBNP levels in individuals suffering from heart failure.

For sepsis and vehicle groups, the majority of the histopathological damage scores were quite severe (score 3). On the other hand, when compared to sepsis and vehicle groups, atorvastatin treatment dramatically minimizes cardiac tissue injury. The group that received atorvastatin showed histopathological damage levels that were mild (score 1). 25 showed that an atorvastatin pretreatment significantly reduced neutrophil accumulation and oxidative stress. When taking atorvastatin, reduced glutathione levels seemed to return to a roughly controlled level. Additionally, the pretreatment reduced histopathological-level mucosal damage 26

found that administering atorvastatin intravenously during ischemia lowers senescence, apoptosis, and Cardioprotective / metabolic, associated indicators. Demonstrated that H and E-stained slices from the Atorvastatin group of male albino rat showed cardiomyocytes with characteristic striated branching architecture, acidophilic cytoplasm, central oval vesicular nuclei, and interstitial blood capillaries.

Conclusion

The study's results confirm the concept that Atorvastatin possesses notable anti-apoptotic and Cardioprotective characteristics, effectively reducing cardiac harm caused by sepsis.

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