International Journal of Pharmacology

ISSN 1811-7775

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2021.577.583

Research Article
Involvement of BDNF Signalling Pathway in Spironolactone-Mediated Protective Effects in Sepsis-Induced Cardiac Injury in Rats

Jiajiu Yao, Zhen Qian, Xiaoxi Tian, Guoqiang Fu, Boliang Wang and Lihong Li

Department of Emergency Medicine, Tangdu Hospital, Air Force Military Medical University, No. 1 Xinsi Road, Baqiao, Xi'an, Shaanxi, 710038, China

Abstract

Background and Objective: Sepsis is one of the leading causes of mortality and myocardial injury is one of the major outcomes of sepsis. Recently, the renin-angiotensin-aldosterone modulating drug is reported to be useful in sepsis-induced cardiac injury. It is possible that spironolactone, an aldosterone receptor blocker, may attenuate sepsis-induced cardiac injury. The present study explored the beneficial effect and molecular mechanisms involved in spironolactone-mediated effects in sepsis-induced cardiac injury. **Materials and Methods:** Lipopolysaccharide (LPS) was employed to induce sepsis and cardiac injury in Wistar rats. The extent of myocardial injury was assessed by measuring the levels of cardiac troponin (cTnT) and CK-MB in plasma. The levels of inflammatory markers, IL-1 and anti-inflammatory, IL-10 were assessed as markers of inflammation. Treatment with spironolactone (25 and 50 mg kg^{-1}) was done in sepsis-subjected rats. The levels of BDNF were measured to explore the molecular mechanism of spironolactone. The role of BDNF was further assessed by treating with BDNF blocker i.e. ANA-12 in spironolactone-treated rats. **Results:** Treatment with spironolactone significantly attenuated the sepsis-induced increase in cTnT and CK-MB levels in a dose-dependent manner. Moreover, it attenuated IL-1 and increased the levels of IL-10 in LPS-injected rats. It also restored a sepsis-induced decrease in the BDNF levels suggesting the key role of BDNF in spironolactone-mediated beneficial effects. Co-administration of ANA-12 abolished the inflammatory actions of spironolactone and protective effects in the sepsis-induced heart injury model. **Conclusion:** Spironolactone may be effective in attenuating sepsis-induced cardiac injury and its protective effects may be possibly due to due to increase in the BDNF levels.

Key words: Aldosterone, BDNF, inflammation, sepsis, heart, spironolactone, pathogenesis, cardiac injury

Citation: Yao, J., Z. Qian, X. Tian, G. Fu, B. Wang and L. Li, 2021. Involvement of BDNF signalling pathway in spironolactone-mediated protective effects in sepsis-induced cardiac injury in rats. Int. J. Pharmacol., 17: 577-583.

Corresponding Author: Lihong Li, Department of Emergency Medicine, Tangdu Hospital, Air Force Military Medical University, No. 1 Xinsi Road, Baqiao, Xi'an, Shaanxi, 710038, China Tel: +8613892860126

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sepsis is one of the leading causes of mortality and myocardial injury is one of the major outcomes of sepsis[1]. However, there is no specific drug currently available to prevent and manage sepsis-induced organ failure including cardiac injury. Recently, renin-angiotensin-aldosterone modulating drug i.e. telmisartan has been reported to be useful in sepsis-induced cardiac injury[2]. Therefore, it is possible that another drug that modulates the renin-angiotensin-aldosterone modulating such as spironolactone, an aldosterone receptor blocker, may attenuate sepsis-induced cardiac injury.

Spironolactone is a mild diuretic and it has been widely used clinically due to its antifibrotic actions in congestive heart failure[3]. Furthermore, it has also been shown that aldosterone contributes to initiating and maintaining inflammation[4] and aldosterone receptor blockers have the potential to attenuate inflammation[5]. Owing to inflammation attenuating actions, it has been shown that spironolactone is useful in several diseases in which inflammation constitutes an important factor in pathogenesis[6-8]. Since systemic inflammation is a critical factor in inducing cardiac injury during sepsis[9,10], therefore, spironolactone may be useful in attenuating cardiac injury in the sepsis-model due to its potent anti-inflammatory actions.

Brain-Derived Neurotrophic Factor (BDNF) is a factor in the body and has been shown to exert multiple actions including protection from sepsis-induced cardiac injury[11]. Moreover, spironolactone has been shown to increase the expression of BDNF and its usefulness has been possibly attributed to an increase in BDNF levels[12]. Therefore, it may be possible that spironolactone may produce beneficial effects in sepsis-induced cardiac injury by increasing BDNF levels. Based on these, the present study was designed to explore the beneficial effect and molecular mechanisms involved in spironolactone-mediated effects in sepsis-induced cardiac injury models in rats.

MATERIALS AND METHODS

Study area: The experiments were performed in the Department of Emergency Medicine, Tangdu Hospital, Air Force Military Medical University, No. 1 Xinsi Road, Baqiao, Xi'an, Shaanxi, 710038, China between February-July, 2021.

Animals and chemicals: In this study, forty-eight Male Wistar albino rats (200-250 g) were used. All these animals were kept in the standard laboratory conditions in the Animal House of the department. The experimental protocol was approved by the Institutional Ethical Committee of Department of Emergency Medicine, Tangdu Hospital, Air Force Military Medical University, No.1 Xinsi Road, Baqiao, Xi'an, Shaanxi, 710038, China with approval number: IACUC: 20210802.

LPS-induced sepsis model: LPS was injected intraperitoneally in rats at the dose of 4 mg kg^{-1} and after 6 hrs of injection, cardiac injury parameters and other biochemical parameters were assessed[13].

Assessment of cardiac injury parameters: The assessment of heart injury is usually done by measuring the levels of heart-specific biomarkers in the circulation. The cardiac troponin (cTnT) and Creatine Kinase (CK-MB) serve as specific biomarkers of heart injury and hence, their levels were estimated to assess the degree of heart injury in response to induction of sepsis[14,15]. The levels of cardiac injury biomarkers i.e. cardiac troponin (cTnT) and Creatine Kinase (CK-MB) were assessed in the plasma. The levels of cTnT and CK-MB were estimated by using commercially available assay kits and their estimations were performed using instructions manual.

Assessment of other biochemical parameters: Systemic inflammation is characterized by an increase in the levels of pro-inflammatory mediator IL-1 and a decrease in the levels of anti-inflammatory mediator IL-10. Therefore, in the present study, their levels were quantified to assess the LPS-induced extent of systemic inflammation. The levels of IL-1 and IL-10 were assessed in the plasma using a commercially available ELISA kit and estimations were performed as per instructions on manual. The levels of BDNF were estimated in the heart homogenates. The hearts were removed and homogenised in Phosphate Buffer Solution (PBS), which was then centrifuged at 5000 g at 4°C for 15 min to obtain homogenate. The levels of BDNF were estimated in heart homogenates using commercially available ELISA kits and estimations were done as per instructions of the manual.

Experimental protocol: There were six groups and eight rats in each group. These groups included group I (normal) in which no intervention was given and biochemical parameters were performed; group II (LPS) in which LPS was given (4 mg kg^{-1}, i.p.) and different biochemical parameters were assessed after 6 hrs of injection, groups III and IV in which spironolactone 25 or 50 mg kg^{-1} was given three days before LPS injection and was also given on the day of LPS injection, groups V and VI in which ANA-12 (0.25 or 0.5 mg kg^{-1}) was co-administered along with

spironolactone (50 mg kg^{-1}) for three days before LPS injection and also on the day of LPS injection.

Statistical analysis: The data of the present study was represented in the form of Mean SD. The statistical analysis of data was done using One way ANOVA followed by Tukey's *post hoc* test. The value of $p<0.05$ was considered statistically significant.

RESULTS

Effect of different pharmacological agents on cardiac injury parameters: Injection of LPS in rats led to significant myocardial injury as there was a significant increase in the levels of cardiac troponin (cTnT) (Fig. 1) and heart-specific isoform of creatine kinase (CK-MB) (Fig. 2) in the plasma. Treatment with spironolactone (25 and 50 mg kg^{-1})

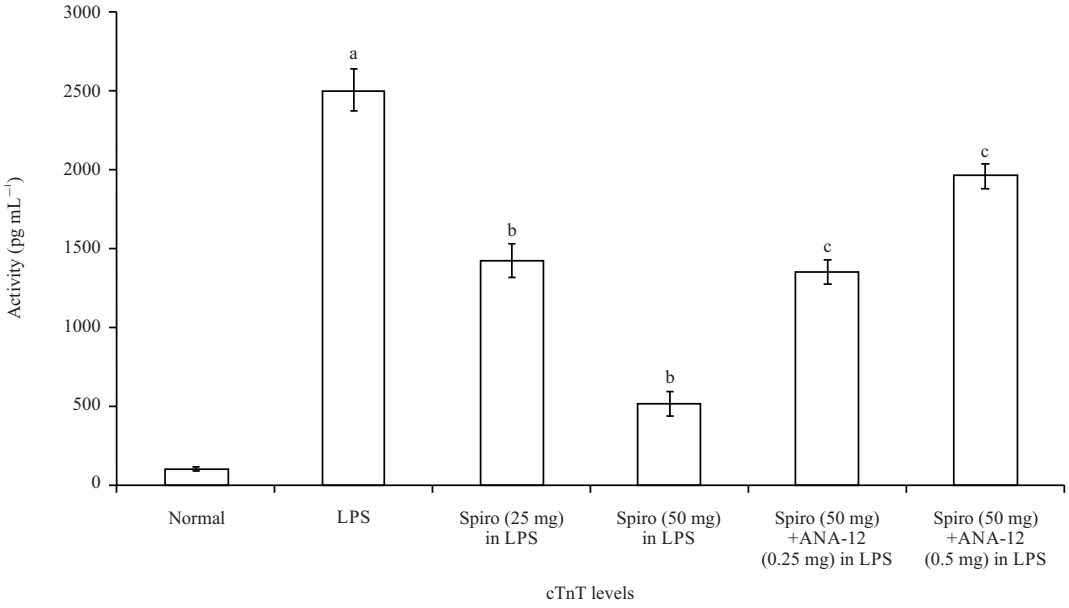

Fig. 1: Effect of spironolactone and ANA-12 in LPS-induced cardiac injury in terms of increase in the release of cTnT in the plasma
Values are in Mean±SD, [a]p<0.05 vs. normal, [b]p<0.05 vs. LPS, [c]p<0.05 vs. Spiro (50 mg kg^{-1}), LPS: Lipopolysaccharide, Spiro: Spironolactone

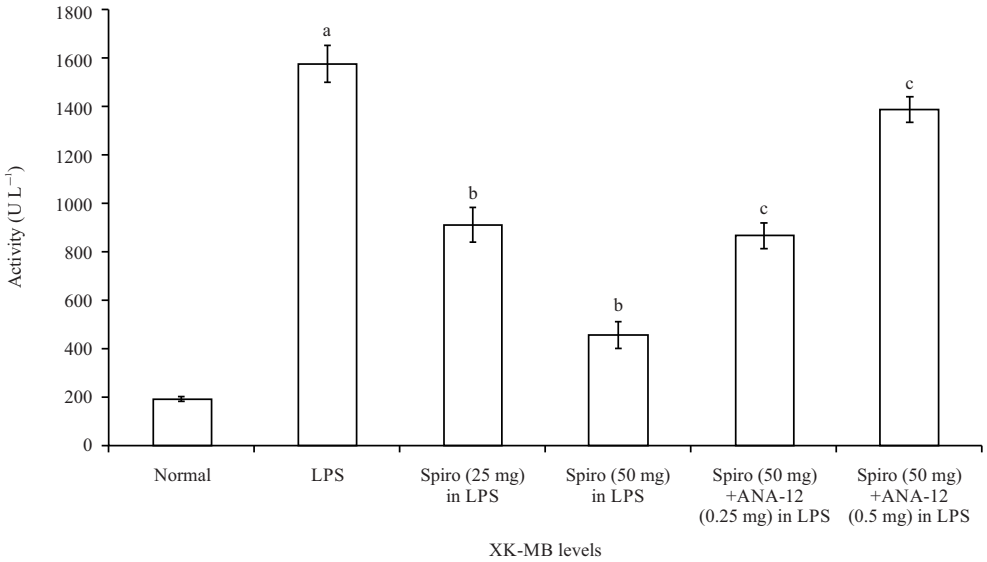

Fig. 2: Effect of spironolactone and ANA-12 in LPS-induced cardiac injury in terms of increase in the release of CK-MB in the plasma
Values are in Mean±SD, [a]p<0.05 vs. normal, [b]p<0.05 vs. LPS, [c]p<0.05 vs. Spiro (50 mg kg^{-1}), LPS: Lipopolysaccharide, Spiro: Spironolactone

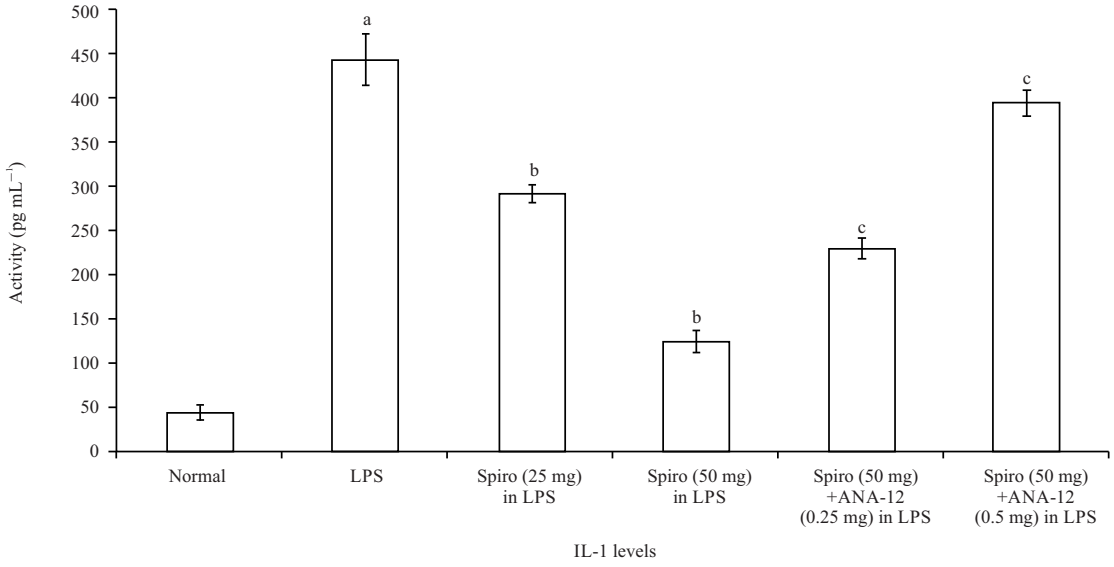

Fig. 3: Effect of spironolactone and ANA-12 in LPS-induced inflammation in terms of increase in the levels of IL-1 in the plasma
Values are in Mean±SD, [a]p<0.05 vs. normal, [b]p<0.05 vs. LPS, [c]p<0.05 vs. Spiro (50 mg kg^{-1}), LPS: Lipopolysaccharide, Spiro: Spironolactone

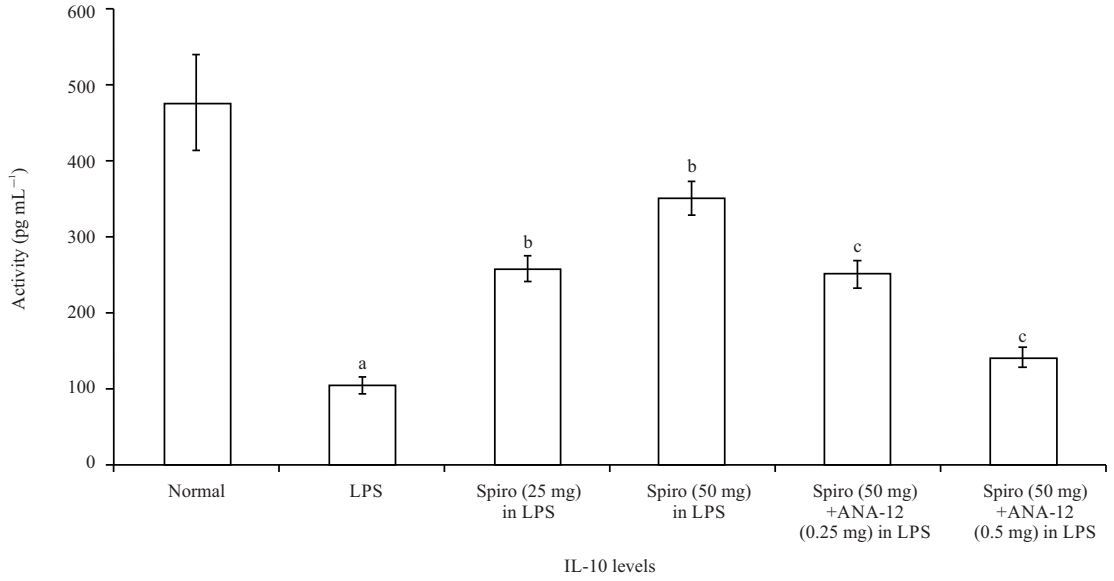

Fig. 4: Effect of spironolactone and ANA-12 in LPS-induced inflammation in terms of decrease in the IL-10 levels in the plasma
Values are in Mean±SD, [a]p<0.05 vs. normal, [b]p<0.05 vs. LPS, [c]p<0.05 vs. Spiro (50 mg kg^{-1}), LPS: Lipopolysaccharide, Spiro: Spironolactone

significantly attenuated LPS-induced myocardial injury and there was normalization of the levels of cTnT and CK-MB. However, administration of ANA-12 (0.25 and 0.50 mg kg^{-1}), BDNF blocker, along spironolactone (50 mg kg^{-1}) abolished the beneficial effects of the latter and there was a rise in cTnT and CK-MB levels in BDNF blocker-treated groups.

Effect of different pharmacological agents on inflammatory markers: Injection of LPS in rats led to a significant increase in systemic inflammation as there was a significant increase in the levels of proinflammatory mediator, IL-1 (Fig. 3) and a decrease in an anti-inflammatory mediator, IL-10 (Fig. 4) in the plasma. Treatment with spironolactone (25 and 50 mg kg^{-1}) significantly attenuated LPS-induced increase in inflammatory markers and there was normalization of the levels of IL-1 and IL-10. However, administration of ANA-12 (0.25 and 0.50 mg kg^{-1}), BDNF blocker, along spironolactone (50 mg kg^{-1}) abolished the beneficial effects of the latter on

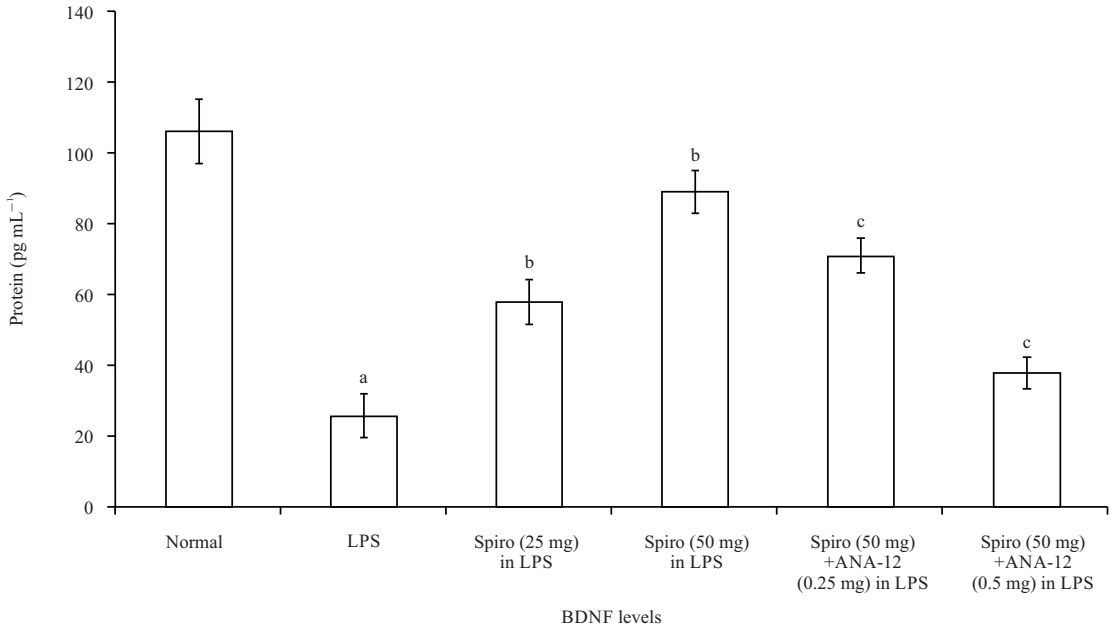

Fig. 5: Effect of spironolactone and ANA-12 in LPS-induced changes in the BDNF levels in the heart homogenates
Values are in Mean±SD, [a]p<0.05 vs. normal, [b]p<0.05 vs. LPS, [c]p<0.05 vs. Spiro (50 mg kg^{-1}), LPS: Lipopolysaccharide, Spiro: Spironolactone

inflammation and there was a rise in IL-1 and decline in IL-10 levels in BDNF blocker-treated groups.

Effect of different pharmacological agents on BDNF levels in the heart: Injection of LPS in rats led to a significant decrease in the BDNF levels in the heart (Fig. 5). Treatment with spironolactone (25 and 50 mg kg^{-1}) significantly restored the BDNF levels in the heart in a dose-dependent manner. However, administration of ANA-12 (0.25 and 0.50 mg kg^{-1}), BDNF blocker, along with spironolactone (50 mg kg^{-1}) abolished the beneficial effects of latter on restoring the BDNF levels and there was a significant decrease in the levels of BNF in LPS-injected rats.

DISCUSSION

In this study, intraperitoneal injection of LPS led to significant induction of sepsis and sepsis-induced heart injury, which was assessed by measuring the levels of cTnT and CK-MB in the plasma. The high circulating levels of and cTnT and CK-MB indicates the significant induction of heart injury. LPS is one of the most common pharmacological agents to induce sepsis in rodents[16,17] and LPS-induced sepsis closely mimics sepsis in humans[18]. There have been previous studies showing that LPS-induced sepsis can produce widespread deleterious effects including on the heart and published reports have shown the significant heart injury in LPS-injected rodents[19]. Therefore, the present study results showing the induction of heart injury in response to LPS-injection are in consonance with previously published studies showing heart injury in LPS-induced sepsis models.

In the study, treatment with spironolactone (25 and 50 mg kg^{-1}) for four days (three days before LPS and on a day of LPS injection) significantly prevented LPS-induced heart injury in a dose-dependent manner. In spironolactone-treated rats, there was a significant decrease in the release of cTnT and CK-MB from the heart to the plasma suggesting the cardioprotective actions of spironolactone in sepsis-induced heart injury. Spironolactone is an aldosterone antagonist and has been employed clinically in the management of congestive heart failure[20]. There have been studies showing the widespread effectiveness of spironolactone in various preclinical studies including brain injury[21], heart injury[22], intestinal injury or lung injury[23]. However, to the best of our knowledge, it is the first study showing the effectiveness of spironolactone in attenuating sepsis-induced cardiac injury in rats.

In the present study, there was also an increase in the levels of IL-1 (a pro-inflammatory cytokine) and a decrease in the levels of IL-10 (anti-inflammatory cytokine) in LPS-injected rats. It suggests that injection of LPS led to significant induction of inflammatory response, which may be responsible for heart injury. There have been previous studies

showing that LPS-injection increases inflammatory response, which may contribute to myocardial injury[24,25]. However, treatment with spironolactone significantly attenuated LPS-induced increase in inflammatory response as there was a decrease in the levels of IL-1 and an increase in the levels of IL-10. There have been previous studies showing that spironolactone exerts an anti-inflammatory response and its beneficial effects in many disease states is attributed to its anti-inflammatory reactions[26]. Since inflammatory reactions are critical to heart injury in sepsis model[27,28] and spironolactone is capable of reducing inflammation, therefore, it is possible to suggest that spironolactone-mediated inflammatory reactions may contribute to attenuating sepsis-induced myocardial injury.

In the present study, there was a significant decrease in the levels of BDNF in the heart homogenates in sepsis-induced heart injury. There have been previous studies showing that a decrease in the levels of BDNF in the sepsis model in rats[11]. Treatment with spironolactone restored the levels of BDNF in the heart in LPS-injected rats in a dose-dependent manner. There have been previous studies showing that spironolactone is capable of increasing the expression of BDNF[12]. Therefore, spironolactone may increase the expression of BDNF to prevent myocardial injury in LPS-injected rats. The role of BDNF in spironolactone-mediated protective effects in sepsis-induced myocardial injury was further supported by the results of the present study showing that co-administration of ANA-12, BDNF blocker, attenuated the cardioprotective effects of spironolactone. Furthermore, ANA-12 also attenuated the spironolactone-mediated decrease in inflammatory response and restoration of BDNF levels. Therefore, it may be proposed that spironolactone restores the expression of BDNF to attenuate inflammatory reaction, which may participate in decreasing heart injury in sepsis-induced heart injury.

CONCLUSION

The study provides evidence that aldosterone receptor antagonist, spironolactone may be effective in attenuating sepsis-induced heart injury. The cardioprotective effects of spironolactone in the sepsis model may be possibly due to the restoration of BDNF levels. The increase in the BDNF levels may, in turn, attenuate the inflammatory cascade to result in a decrease in the release of inflammatory mediators including IL-1 and an increase in the release of anti-inflammatory mediators, IL-10. Spironolactone-mediated increase in the levels of BDNF and decrease in inflammation may help protect the heart from sepsis-induced injury.

SIGNIFICANCE STATEMENT

The study discovers the potential of an aldosterone antagonist, spironolactone in protecting the heart from sepsis-induced injury. Till now, researchers were able to explore the usefulness of spironolactone in other areas of injury. However, this is the first study to un-reveal the effectiveness of spironolactone in sepsis-induced cardiac injury in rats. The study will help the researchers to further explore the molecular mechanisms involved in spironolactone-mediated restorative effects on BDNF and decrease in inflammatory cascade in the sepsis model.

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