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Research Article

Amantadine preserve VCAM immunoexpression levels in cardiac injury induced by brain trauma with its anti-inflammatory action and protective effect on the mitochondrial membrane

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ABSTRACT

Brain trauma-induced cytokine storms can impact multiple organs, particularly the heart, through inflammatory and apoptotic mechanisms. This study aimed to examine cardiac pathology following experimental brain trauma (BTCI) and evaluate the protective effects of amantadine (AMN), an NMDA receptor antagonist, on cardiotoxicity. Forty rats were divided into four groups: sham, BTCI, BTCI+AMN-1 (45 mg/kg, ip), and BTCI+AMN-7. Trauma (0.2 Newton) was induced by dropping a 50 g ball from 80 cm. Heart samples were collected 24 hours and seven days post-trauma for histopathological and immunohistochemical analysis. The BTCI group showed hyperemia, hemorrhage, inflammatory infiltrations, increased Bax and VCAM expressions, and decreased Bcl-2 expression. AMN treatment reversed these findings, with greater efficacy observed after seven days. In conclusion, BTCI induces cardiac damage, while AMN provides protective effects. Further studies are needed to clarify underlying mechanisms.

Key words: Brain trauma, heart, VCAM, Bax, Bcl2, inflammation

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INTRODUCTION

In brain trauma caused by various reasons, there is a large amount of cytokine production and release in the tissue as a result of damage to the skull base. It has been proven that these cytokines mixed with the blood can also cause damage to peripheral organs through the permeability of the blood-brain barrier (Cardona et al., 2003; Wilson et al., 2010; Gyoneva and Ransohoff, 2015). Chemokines are released by injured neuronal tissue after brain injury, drawing immune cells to the area of harm. Different cellular processes may be involved in the ensuing inflammatory response, depending on the kind of insult—traumatic contusion, diffuse damage, or elevated intracranial pressure. Experimental trauma studies have shown that in injured areas, neutrophil infiltration occurs first, peaking within a few days. Following this, astrocytes, lymphocytes, and microglia/macrophages migrate to the site of injury (Cardona et al., 2003; Wilson et al., 2010; Gyoneva and Ransohoff, 2015; Alam et al., 2020). It is known that the heart tissue, which is responsible for the blood supply of the whole body, is also affected by this damage, which can indirectly cause an imbalance in the oxygenation of the organs and deepen the damage (Morganti-Kossman et al., 1997; Cardona et al., 2003; Wilson et al., 2010; Gyoneva and Ransohoff, 2015; Alam et al., 2020).

Over the past 20 years, studies in humans have reported that cardiovascular complications have become

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more widespread after brain injury and are associated with increased morbidity and mortality. Hypertension, hypotension, cardiac arrhythmias, ECG abnormalities, the production of biomarkers of cardiac damage, and left ventricular dysfunction are among the range of abnormalities (Zygun, 2005; van der Bilt et al., 2009; Mueller et al., 2020; Huang et al., 2023; Coppalini et al., 2024). In order for cytokines circulating in the blood to affect and penetrate tissues, the synthesis of vascular cell adhesion molecules (VCAM) in the endothelial layer increases. It is known that cytokines that adhere to tissues via these molecules trigger damage, primarily inflammation, in the tissue and also affect apoptotic processes. VCAM-1 has been reported to be an important biomarker in cardiovascular diseases (Troncoso et al,.2021). It has been proven that apoptosis occurring in cardiac tissue can cause losses at the cellular level, leading to deterioration in functions and indirectly in the vascularization of distant organs. It has been shown that in the event of damage to mitochondria, which are responsible for energy and respiration at the cellular level within the cell, B-cell lymphoma 2 (BCL-2) and Bcl-2-associated X protein (BAX) levels are disrupted, causing apoptosis (Bennett, 2002; Elmore 2007).

It is known that amantadine has antiviral effects against influenza A virus, reduces cytotoxicity with its N-methyl-D-aspartate (NMDA) receptor antagonist effect, and causes dopamine discharge in the treatment of parkinsonism. It has been shown in a limited number of studies to have anti-inflammatory and antiapoptotic effects (Jiménez-Jiménez et al., 2020; Dekundy, et al., 2024).

Publications reporting cardiac manifestations following brain trauma have rapidly increased in recent years. However, experimental studies and explanations regarding the pathogenetic mechanisms remain quite limited. The aim of this study is to investigate the pathological and immunohistochemical changes occurring in the heart after experimental brain trauma (BTCI), and to explore the effects of amantadine on VCAM/BCL2 and BAX expressions in its potential protective role against cardiac damage induced by brain trauma. The potential action mechanism of brain trauma induced heart injury is shown in Figure 1.

MATERIAL AND METHODS

Animals and ethical approval

All experimental processes applied in the study was performed following the guidelines for animal research outlined by the Animal Research: Reporting In Vivo Experiments (ARRIVE) 2.0 and were approved by the Committee on Animal Research at Süleyman Demirel University (approval no: 392). Furthermore, support for the study was provided by the Süleyman Demirel University Scientific Research Project Unit (SDU-BAP) under the project number TSG-2023-9092. During the trials, the animals from Süleyman Demirel University's Animal Experiments Laboratory were kept at 21–22°C, 60% ± 5% humidity, and a 12-hour light–12-hour dark cycle. They were also given water and standard commercial feed as needed.

Creation of trauma model

In order to create a brain trauma model, the impact acceleration model made by Marmarou et al. by dropping a 50 mg ball from a height of 80 cm was used. Thus, it was aimed to create a trauma of 0.2 N severity according to Newton's law (Marmarou et al., 1994)

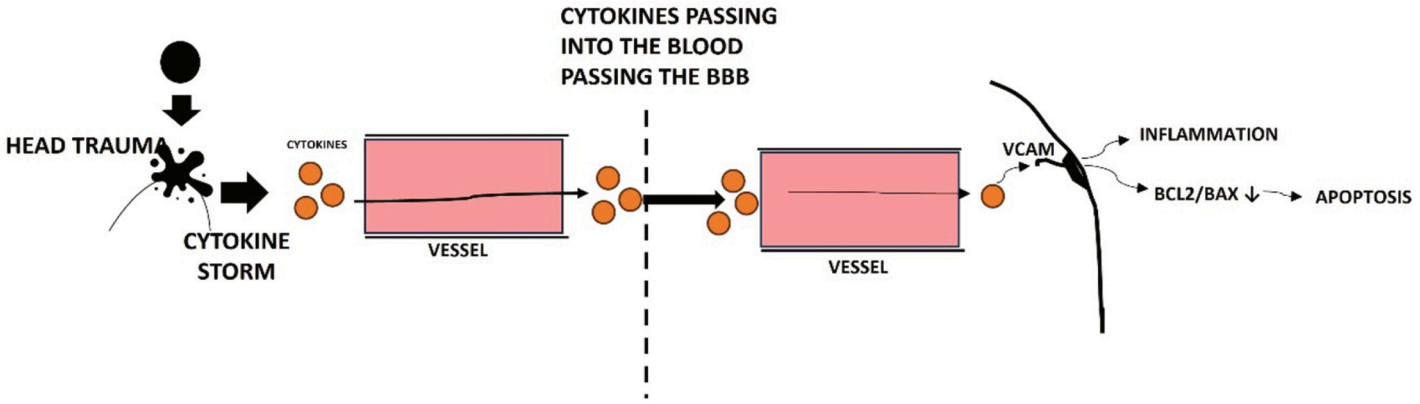

Figure 1. Potential pathological mechanism of brain trauma induced cardiac injury
VCAM: Vascular cell adhesion molecules, BCL2: B-cell lymphoma 2, BAX: Bcl-2-associated X protein

STUDY DESIGN

Totally, weighing 300-350 grams forty Wistar Albino male were divided into four groups. Groups were;

- Sham group: Incision was made and trauma was not induced. Then 0.5-1 ml of physiological serum (SF) were administered intraperitoneally (ip). After 24 hours, rats sacrificed under anesthesia and heart tissues were taken.
- BTCI group: Incision were made, trauma was created and then 0.5-1 ml of SF administered ip. After 24 hours, rats sacrificed under anesthesia and heart tissues were taken.
- BTCI+AMN-1: Incision were made, trauma was created and then 0.5-1 ml 45 mg/kg AMN were administered ip (Hardeland et al., 2010; Orhan et al., 2021). After 24 hours, rats were sacrificed under anesthesia and heart tissues were taken.
- BTCI+AMN-7: Incision were made, trauma was created and then 45 mg/kg AMN were administered ip once a day for 7 days. After the 7th day, the rats sacrificed under anesthesia and their heart tissues were taken.

For all experimental procedures 80-90 mg/kg ketamine (Ketalar, Pfizer, Türkiye) and 8-10 mg/kg xylazine (Xylazinbio %2, Bioveta, Czech Republic) were used for anesthesia. A surgical exsanguination was performed by taking blood from the inferior vena cava for the sacrification process. Blood samples and heart tissue were taken and fixed in formaldehyde solution for histopathological and immunohistochemical examinations.

Histopathological Method

Heart samples were taken during necropsy and fixed in a 10% neutral formalin solution. Afterwards, the samples were embedded in paraffin wax using a standard tissue processing method employing a fully automated tissue processing device (Leica ASP300S, Wetzlar, Germany). Subsequently, 5 µm thick sections were sliced from the paraffin blocks using a fully automated rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). These sections were then stained with hematoxylin-eosin (HE), covered, and examined under light microscopy.

Histological lesions in the hearts were assessed semi-quantitatively using an ordinal grading system. This assessment involved evaluating hyperemia, hemorrhage, inflammatory cell infiltrations, and degenerative necrotic changes in myocardial cells. Descriptions of normal (score = 0), mild (score = 1), moderate (score = 2), and severe (score = 3) affections were assigned scores ranging from 0 to 3 (Table 1).

Table 1: Histopathology score of hepatic lesions

	Severity	Description	Score
Histopathological changes	Normal		0
	Mild	< 3 foci	1
	Marked	4-6 foci	2
	Severe	> 7 foci	3

Immunohistochemical examination

Three sets of slices were cut from the paraffin blocks and placed on poly-L-lysine-coated slides for immunohistochemical examination. Following the manufacturer's instructions, sections were then immunohistochemically stained using the streptavidin-biotin method to evaluate the expression of Bax (Recombinant Anti-Bax antibody [E63] (ab32503)), Bcl-2 (Anti-Bcl-2 antibody (ab194583)), and VCAM (Recombinant Anti-VCAM1 antibody [EPR5047] (ab134047)). Abcam (Cambridge, UK) supplied the primary antibodies, which were utilized at a dilution of 1/100. The sections were incubated with the primary antibodies for 60 minutes before being subjected to immunohistochemistry using a biotinylated secondary antibody and a streptavidin-alkaline phosphatase conjugate. The chromogen was diaminobenzidine (DAB), and the secondary antibody was the Mouse and Rabbit Specific HRP/DAB IHC Detection Kit-micro-polymer (ab236466) from Abcam (Cambridge, UK). Primary antibodies were substituted with antigen dilution solution for negative controls. A qualified pathologist from a different university performed each evaluation on blinded samples.

At an objective magnification of X40, the IHC expressions were scored on a scale of 0-3. Accordingly, 0 indicates no expression, 1 indicates focal and weak staining, 2 indicates diffuse and weak staining, and 3 indicates diffuse and marked staining (Table 2). The Image J 1.48 software (National Institutes of Health, Bethesda MD) was used to determine the positive

reaction. An Olympus CX41 model microscope was used for photographing the results, and the Database Manual Cell Sens Life Science Imaging Software System

(Olympus Corporation, Tokyo, Japan) was used for microphotography.

Table 2: Immunohistochemical score of cardiac biomarkers

Immunohistochemistry expressions	Focal and weak staining	1
	Diffuse and weak staining	2
	Diffuse and marked staining	3

Statistical Analysis

We utilized Graphpad Prism 10 (Version 10.1.0) (GraphPad Software, USA) for statistical analysis. The Shapiro-Wilk test was first used to examine the data for normality of distribution. One-way analysis of variance (ANOVA) was used to compare the groups because the data displayed a normal distribution (P>0.05). Group differences were determined using the Tukey test, with P < 0.05 being regarded as statistically significant. The Mann-Whitney U test and Dunnett's C test were employed to identify group differences in nonparametric data.

RESULTS

Histopathological findings

Microscopic examination revealed no pathological changes in the myocardial tissue of the control group. Cardiomyocytes in this groups appeared elongated, branching, and of normal size with well-defined intercalated discs. Delicate endomysium sheaths surrounding the cardiac cells were observed, along with a dense capillary network surrounding the cells. In contrast, the BTCI group exhibited alterations in cardiac tissue, including hyperemia, hemorrhage, and inflammatory cell infiltrations. Degenerative changes characterized by an eosinophilic appearance were observed in cardiomyocytes. Treatment with AMN resulted in the amelioration of these pathological findings. Seven days of treatment were more effective than one day of treatment (Figure 1).

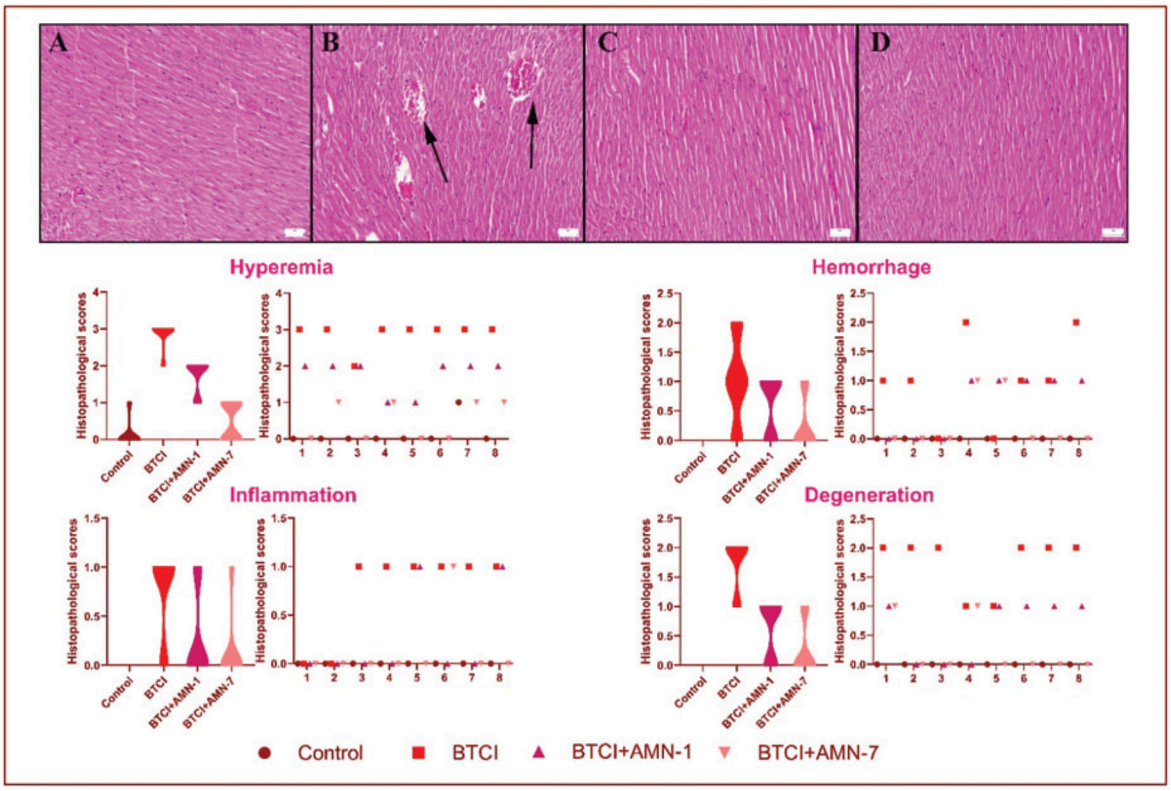

Figure 1: Representative histopathological images of hearts across the groups. (A) Normal myocardial tissue histology in the control group, (B) Severe hyperemia (arrows) in the BTCI group, (C) Marked amelioration in pathological changes in the BTCI+AMN-1-day group, (D) Normal myocardium histology in the BTCI+AMN 7-day group, E, scale bars=20µm. BTCI: Brain trauma induced cardiac injury, AMN: Amantadine, Values are represented as means ± SD, p≤0.001.

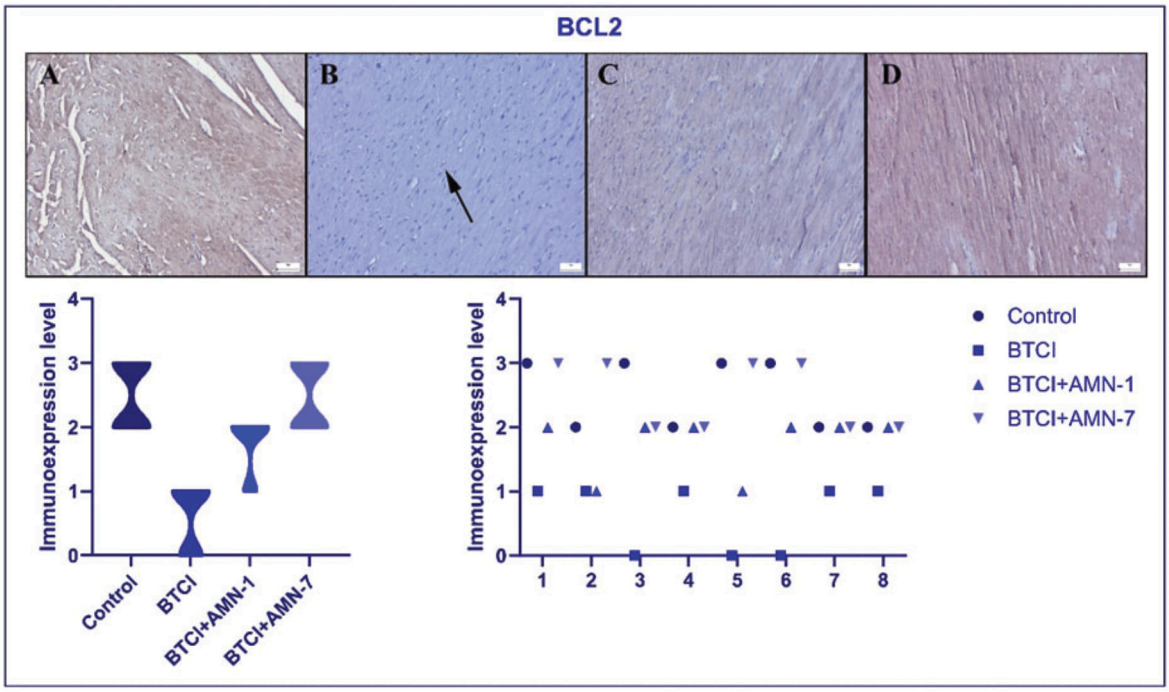

Figure 2. Immunohistochemical expressions of Bcl-2 in the hearts across the groups. (A) Marked Bcl-2 expression in the control group. (B) Decreased Bcl-2 expression in myocardial cells (arrows) in the BTCI group. (C) Moderately increased Bcl-2 expression in the BTCI+AMN-1-day group. (D) Marked Bcl-2 expression in the BTCI-AMN-7-day group, Streptavidin biotin peroxidase method, scale bars = 20 μm. Bcl-2: B-Cell Lymphoma 2, BTCI: Brain trauma induced cardiac injury, AMN: Amantadine, Values are represented as means ± SD, p≤0.001.

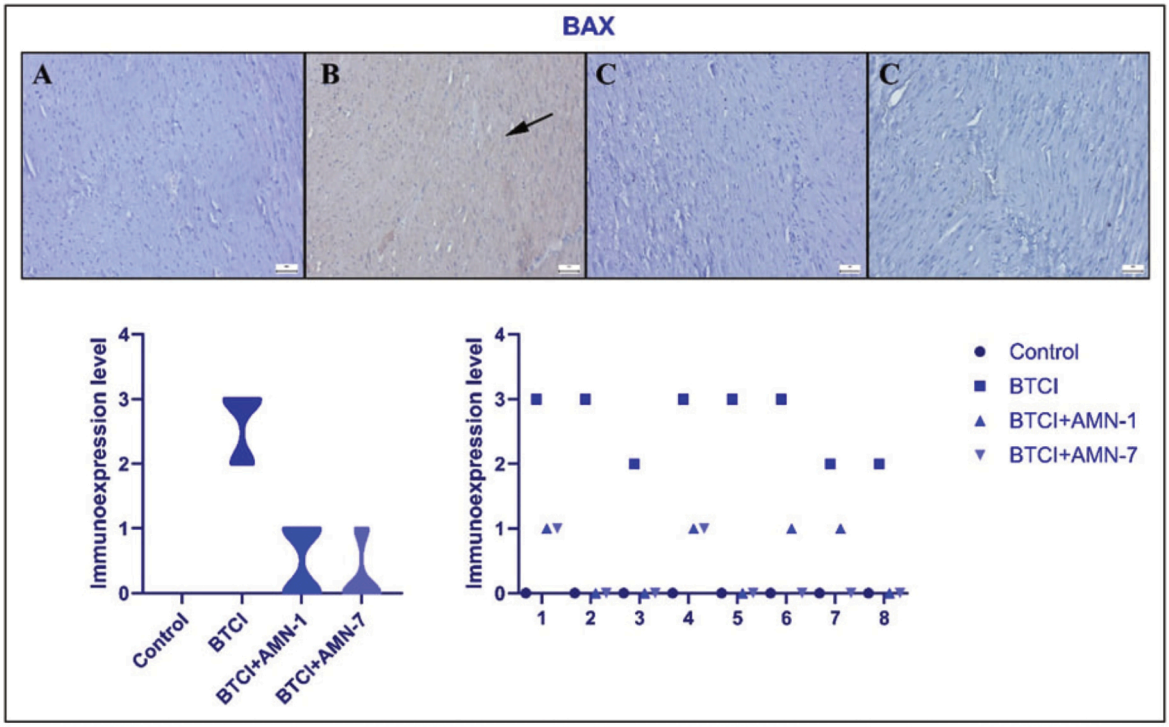

Figure 3. Immunohistochemical expressions of Bax in the hearts across the groups. (A) Negative Bax expression in the control group. (B) Increased Bax expression in myocardial cells (arrows) in the BTCI group. (C) Markedly decreased Bax expression in the BTCI+AMN-1-day group. (D) Negative Bax in the BTCI-AMN-7-day group, Streptavidin biotin peroxidase method, scale bars = 20 μm. Bax: Bcl-2-Associated X Protein, BTCI: Brain trauma induced cardiac injury, AMN: Amantadine, Values are represented as means ± SD, p≤0.001.

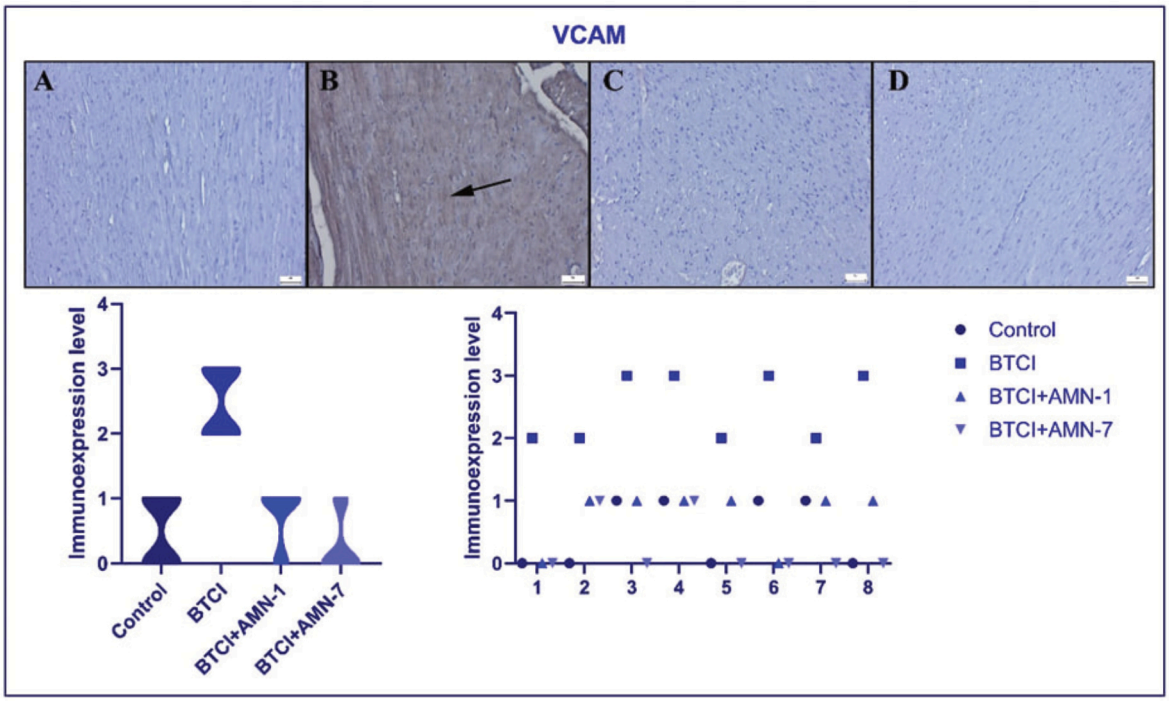

Figure 4. Immunohistochemical expressions of VCAM in the hearts across the groups. (A) Negative VCAM expression in the control group. (B) Increased VCAM expression in myocardial cells (arrows) in the BTCI group. (C) Markedly decreased VCAM expression in the BTCI+AMN-1-day group. (D) Negative VCAM expression in the BTCI-AMN-7-day group, Streptavidin biotin peroxidase method, scale bars = 20 µm. VCAM: Vascular cell adhesion molecule, BTCI: Brain trauma induced cardiac injury, AMN: Amantadine, Values are represented as means ± SD. *p≤0.05, **p≤0.01, ***p≤0.001.)

Immunohistochemical examination

Immunohistochemical investigations revealed very low or non-existent expression of Bax and VCAM but marked expression of Bcl-2 in the control group. Conversely, myocardial cells in the BTCI group exhibited moderate to markedly increased expressions of Bax and VCAM, along with decreased Bcl-2 expressions. Treatment with AMN reversed these pathological findings. Seven days of treatment were more effective than one day of treatment (Fig. 2-4).

DISCUSSION

In this study, heart damage resulting from brain trauma was experimentally demonstrated in a rodent model. Histopathological and immunohistochemical changes in the heart were assessed to explain the mechanism of heart damage. The study results revealed that following brain trauma, both apoptotic and inflammatory activity increased in the heart, leading to myocardial damage. It was also shown for the first time that AMN reduced this damage and could potentially be used in cases of brain trauma.

It is known that traumas occurring in the human body for many reasons can cause distant organ damage in addition to the parts exposed to the trauma (Rachfalska et al., 2020). For this reason, multiorgan damages that occur are fatal and often require intensive care conditions (Cole et al., 2020; Ting et al., 2023). Basic agents used in intensive care conditions may also be insufficient in the treatment of these multiple damages (Abdelbaky et al., 2024). In this study, acute and chronic applications of AMN were effective in reducing the damage in cardiac damage secondary to brain trauma in experimental animals. Although brain trauma seems to be localized to the damage site, distant organ damage can occur due to the inflammatory picture caused by cytokines released from the damaged area into the blood, passing to the peripheral compartment and reaching other tissues. Inflammatory cytokines circulating in the vessel can trigger damage in those areas by binding to their own receptors in the vascular endothelium and heart tissue (Rachfalska et al., 2020). In parallel with this situation, molecules such as VCAM must be present and their expressions must increase in order for inflammatory cytokines to pass to

tissues outside the vessel such as the heart (Kong et al., 2018). In this study, the increase in VCAM expression in the BTCI group with damage to the heart tissue after the brain trauma model supports this situation. In histopathological analyses, the findings of ligated, branching, and of normal size with well-defined intercalated discs, delicate endomysium sheaths surrounding the cardiac cells and dense capillary network surrounding the cells detected in normal heart tissues were replaced by hyperemia, hemorrhage, inflammatory cell infiltrations and degeneration in the BTCI group. The parallelism of these findings with the increase in VCAM expression supports the above-mentioned situations. The regression of all findings with 1-day and 1-week AMN treatment shows that AMN has an anti-inflammatory effect.

Inflammation can often be seen together with apoptosis. For example, it is known that tumor necrosis factor alpha, an indicator of acute inflammation, can trigger apoptosis by directly stimulating caspase-8 mediated caspase-3 by stimulating its receptors on the cell surface, while on the other hand, it can cause damage to the mitochondrial organelle and increase caspase-9 mediated caspase-3 expressions and stimulate apoptosis (Alvarez et al., 2011; McIlwain et al., 2013). Stress, which occurs as a result of damage to the mitochondria, which are responsible for the cell's energy metabolism, causes apoptosis by causing membrane permeability and cytochrome-c release (Guo et al., 2003; Khan et al., 2022; Zong et al., 2024). It is also known that increases in the apoptotic BAX gene found in the membrane are important in cytochrome-c release (Zhang et al., 2017; Garrido et al., 2006). Decreases in this proapoptotic gene with AMN treatment show that apoptosis secondary to inflammation can be regressed by AMN. On the other hand, a decrease in the expression of the BCL gene, which is also an indicator of membrane damage but an antiapoptotic gene, can also trigger apoptosis. The reversal of the decrease in BCL-2 gene expression in the injury groups by AMN shows that the drug has an antiapoptotic attitude (Qian et al., 2022; Öcal et al., 2022).

The limitation of this study is the lack of biochemical and genetic examinations. The primary limitation of this study is the absence of biochemical and genetic analyses. However, this pioneering experimental study has provided valuable insights into the structural and cellular changes in the heart following brain trauma through histopathological and immunohistochemical evaluations.

This study highlights the significant histopathological and immunohistochemical changes in the heart following brain trauma, including increased apoptotic activity and inflammatory responses, which contribute to myocardial damage. The identification of these key mechanisms provides a clearer understanding of the heart's vulnerability to secondary injury post-trauma. Notably, amantadine treatment was shown to effectively reduce both apoptotic and inflammatory markers, indicating its potential as a therapeutic intervention in mitigating myocardial damage in brain trauma cases. These findings underscore the importance of further investigating amantadine's cardioprotective effects in clinical settings.

ETHICAL APPROVAL

All experimental processes applied in the study was performed following the guidelines for animal research outlined by the Animal Research: Reporting In Vivo Experiments (ARRIVE) 2.0 and were approved by the Committee on Animal Research at Süleyman Demirel University (approval no: 392). Furthermore, support for the study was provided by the Süleyman Demirel University Scientific Research Project Unit (SDU-BAP) under the project number TSG-2023-9092. During the trials, the animals from Süleyman Demirel University's Animal Experiments Laboratory were kept at 21–22°C, 60% ± 5% humidity, and a 12-hour light–12-hour dark cycle. They were also given water and standard commercial feed as needed.

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CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

Abdelbaky, A.M., Eldelpshany, M.S., 2024. Intensive care unit (ICU)-related post-traumatic stress disorder: A literature review. Cureus, 16(3), e57049.

Alam, A., Thelin, E.P., Tajsic, T., Khan, D.Z., Khellaf, A., Patani, R., Helmy, A., 2020. Cellular infiltration in traumatic brain injury. Journal of Neuroinflammation, 17(1), 328.

Alvarez, S., Blanco, A., Fresno, M., Muñoz-Fernández, M.Á., 2011. TNF-α contributes to caspase-3 independent apoptosis in neuroblastoma cells: Role of NFAT. PLoS One, 6(1), e16100.

Bennett, M.R., 2002. Apoptosis in the cardiovascular system. Heart 87(5), 480-487.

Cardona, A.E., Gonzalez, P.A., Teale, J.M., 2003. CC chemokines mediate leukocyte trafficking into the central nervous system during murine neurocysticercosis: role of gamma delta T cells in amplification of the host immune response. Infection and Immunity, 71(5), 2634–2642.

Cole, E., Gillespie, S., Vulliamy, P., Brohi, K., 2020. Organ dysfunction in trauma (ORDIT) study collaborators. Multiple organ dysfunction after trauma. The British Journal of Surgery, 107(4), 402-412.

Coppalini, G., Salvagno, M., Peluso, L., Bogossian, E.G., Quispe Cornejo, A., Labbé, V., Annoni, F., Taccone, F.S., 2024. Cardiac injury after traumatic brain injury: clinical consequences and management. Neurocritical Care, 40(2), 477-485.

Dekundy, A., Pichler, G., El Badry, R., Scheschonka, A., Danysz, W., 2024. Amantadine for traumatic brain injury—Supporting evidence and mode of action. Biomedicines, 12(7), 1558.

Dogrul, B.N., Kiliccalan, I., Asci, E.S., Peker, S.C., 2020. Blunt trauma-related chest wall and pulmonary injuries: An overview. Chinese Journal of Traumatology, 23(3), 125-138.

Elmore, S., 2007. Apoptosis: a review of programmed cell death. Toxicologic Pathology, 35(4), 495-516.

Garrido, C., Galluzzi, L., Brunet, M., Puig, P.E., Didelot, C., Kroemer, G., 2006. Mechanisms of cytochrome c release from mitochondria. Cell Death and Differentiation, 13(9), 1423-1433.

Guo, C., Sun, L., Chen, X., Zhang, D., 2013. Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural Regeneration Research, 8(21), 2003-2014.

Gyoneva, S., Ransohoff, R.M., 2015. Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell-cell communication by chemokines. Trends in Pharmacological Sciences, 36, 471-480.

Hardeland, R., 2010. Investigational melatonin receptor agonists. Expert Opinion on Investigational Drugs, 19(6), 747-764.

Huang, C.H., Yang, C.T., Chang, C.C., 2023. Traumatic brain injury and risk of heart failure and coronary heart disease: A nationwide population-based cohort study. PLoS One, 18(12), e0295416.

Jiménez-Jiménez, F.J., Alonso-Navarro, H., García-Martín, E., Agúndez, J.A.G., 2020. Anti-inflammatory effects of amantadine and memantine: Possible therapeutics for the treatment of Covid-19? Journal of Personalized Medicine, 10(4), 217.

Khan, T., Waseem, R., Zehra, Z., Aiman, A., Bhardwaj, P., Ansari, J., Hassan, M.I., Islam, A., 2022. Mitochondrial dysfunction: Pathophysiology and mitochondria-targeted drug delivery approaches. Pharmaceutics, 14(12), 2657.

Kong, D.H., Kim, Y.K., Kim, M.R., Jang, J.H., Lee, S., 2018. Emerging roles of vascular cell adhesion molecule-1 (VCAM-1) in immunological disorders and cancer. International Journal of Molecular Sciences, 19(4), 1057.

Marmarou, A., Foda, M.A., van den Brink, W., Campbell, J., Kita, H., Demetriadou, K., 1994. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. Journal of Neurosurgery, 80(2), 291-300.

McIlwain, D.R., Berger, T., Mak, T.W., 2015. Caspase functions in cell death and disease. Cold Spring Harbor Perspectives in Biology, 7(4), a026716.

Morganti-Kossman, M.C., Lenzlinger, P.M., Hans, V., Stahel, P., Csuka, E., Ammann, E., Stocker, R., Trentz, O.,

Kossmann, T., 1997. Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. Molecular Psychiatry, 2(2), 133-136.

Mueller, K., Thiel, F., Beutner, F., Teren, A., Frisch, S., Ballarini, T., Möller, H.E., Ihle, K., Thiery, J., Schuler, G., Villringer, A., Schroeter, M.L., 2020. Brain damage with heart failure: Cardiac biomarker alterations and gray matter decline. Circulation Research, 126(6), 750-764.

Öcal, Ö., Coşar, A., Nazıroğlu, M., 2022. Amantadine attenuated hypoxia-induced mitochondrial oxidative neurotoxicity, apoptosis, and inflammation via the inhibition of TRPM2 and TRPV4 channels. Molecular Neurobiology, 59(6), 3703-3720.

Orhan, M., Taş Tuna, A., Ünal, Y., Arslan, M., Yazar, H., Sezen, Ş.C., Gözükara, S.I., Palabıyık, O., 2021. The effects of amantadine on lung tissue in lower limb ischemia/reperfusion injury model in rats. Turk Gogus Kalp Damar Cerrahisi Dergisi, 29(1), 77-83.

Qian, S., Wei, Z., Yang, W., Huang, J., Yang, Y., Wang, J., 2022. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Frontiers in Oncology, 12, 985363.

Rachfalska, N., Putowski, Z., Krzych, Ł.J., 2020. Distant organ damage in acute brain injury. Brain Sciences, 10(12), 1019.

Ting, R.S., Lewis, D.P., Yang, K.X., Nguyen, T.A., Sarrami, P., Daniel, L., Hourigan, S., King, K., Lassen, C., Sarrami, M., Ridley, W., Alkhouri, H., Dinh, M., Balogh, Z.J., 2023. Incidence of multiple organ failure in adult polytrauma patients: A systematic review and meta-analysis. The Journal of Trauma and Acute Care Surgery 94(5), 725-734.

Troncoso, M.F., Ortiz-Quintero, J., Garrido-Moreno, V., Sanhueza-Olivares, F., Guerrero-Moncayo, A., Chiong, M., Castro, P.F., García, L., Gabrielli, L., Corbalán, R., Garrido-Olivares, L., Lavandero, S., 2021. VCAM-1 as a predictor biomarker in cardiovascular disease. Biochimica et Biophysica Acta. Molecular Basis of Disease,1867(9), 166170.

van der Bilt, I.A., Hasan, D., Vandertop, W.P., Wilde, A.A., Algra, A., Visser, F.C., Rinkel, G.J., 2009. Impact of cardiac complications on outcome after aneurysmal subarachnoid hemorrhage: a meta-analysis. Neurology, 72, 635-642.

Wenkang, W., Chuanjie, X., Xinglong, M., Xiaoming, Z., Peng, X., 2020. Intensive care unit-acquired weakness: A review of recent progress with a look toward the future. Frontiers in Medicine (Lausanne), 7, 559789.

Wilson, E.H., Weninger, W., Hunter, C.A., 2010. Trafficking of immune cells in the central nervous system. The Journal of Clinical Investigation, 120(5), 1368–1379.

Zong, Y., Li, H., Liao, P., Chen, L., Pan, Y., Zheng, Y., Zhang, C., Liu, D., Zheng, M., Gao, J., 2024. Mitochondrial dysfunction: mechanisms and advances in therapy. Signal Transduction and Targeted Therapy, 9(1), 124.

Zygun, D., 2005. Non-neurological organ dysfunction in neurocritical care: impact on outcome and etiological considerations. Current Opinion in Critical Care, 11, 139-143.