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The Role of Tasimelteon in Modulating Cardiac Injury Following Traumatic Brain Injury: A Focus on Bax/Bcl-2, SIRT1/p53 Signaling, and Inflammatory Cytokine Pathways

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Abstract

Aim: This study explores the cardioprotective effects of Tasimelteon (TASI), a selective melatonin receptor agonist, following traumatic brain injury (TBI). TBI triggers systemic inflammation, contributing to secondary cardiac injury and increased morbidity. While TASI shows neuroprotective properties, its potential to protect the heart after TBI remains unknown.

Methods: Four groups were created from thirty-two adult male rats: Trauma, Trauma + TASI (1 mg/kg), Trauma + TASI (10 mg/kg), and Sham. Heart tissue was taken for genetic, immunohistochemical, and histopathological examinations. Histopathology assessed hyperemia, hemorrhage, inflammation, and necrosis. Immunohistochemistry measured β-tubulin, IL-1, and IL-6 expression, while RT-qPCR analyzed SIRT1, p53, BAX, and BCL-2 mRNA levels.

Results: The Trauma group displayed symptoms of myocardial damage, such as hyperemia, bleeding, and disturbed cell architectures, but the Sham group's histopathological analysis indicated normal myocardial tissue. TASI treatments improved these findings, with TASI-10 being more effective. Immunohistochemistry showed minimal expression of β-tubulin, IL-1, and IL-6 in the Sham group, but significant upregulation in the Trauma group, indicating inflammation. Both TASI treatments reduced these markers, with TASI-10 showing the greatest reduction. According to gene expression study, trauma reduced anti-apoptotic genes (Sirt-1, Bcl-2) and elevated pro-apoptotic genes (Bax, p53). Gene expression was slightly restored by TASI-1, but TASI-10 significantly improved all four genes.

Conclusion: TASI treatment, particularly at a 10 mg dose, effectively ameliorates myocardial injury caused by Trauma, with improvements observed at the histological, molecular, and gene expression levels. This suggests that TASI may hold potential as a therapeutic agent for myocardial protection.

Keywords: Apoptosis, Cardioprotective, Inflammation, Tasimelteon, Traumatic Brain Injury

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Travmatik Beyin Hasarı Sonrası Kardiyak Hasarı Düzenlemede Tasimelteon'un Rolü: Bax/Bcl-2, SIRT1/p53 Sinyalizasyonu ve İnflamatuar Sitokin Yollarına Odaklanma

Öz

Amaç: Bu çalışma, travmatik beyin hasarı (TBI) sonrasında seçici bir melatonin reseptör agonisti olan Tasimelteon (TASİ)'un kardiyoprotektif etkilerini araştırmaktadır. TBI, sistemik inflamasyonu tetikleyerek sekonder kardiyak hasara ve artan morbiditeye katkıda bulunur. TASİ nöroprotektif özellikler gösterse de, TBI sonrasında kalbi koruma potansiyeli bilinmemektedir.

Yöntemler: Otuz iki yetişkin erkek sıçan dört gruba ayrıldı: Sham, Trauma, Trauma + TASİ (1 mg/kg) ve Trauma + TASİ (10 mg/kg). Baş travması, 24 saat sonra, histopatolojik, immünohistokimyasal ve genetik analizler için kalp dokuları toplandı. Histopatoloji hiperemi, hemoraji, inflamasyon ve nekrozu değerlendirdi. İmmünohistokimya β-tubulin, IL-1 ve IL-6 ekspresyonunu ölçerken, RT-qPCR SIRT1, p53, BAX ve BCL-2 mRNA seviyelerini analiz etti.

Bulgular: Histopatolojik inceleme Sham grubunda normal miyokardiyal doku ortaya koyarken, travma grubunda hiperemi, hemoraji ve bozulmuş hücre yapıları gibi miyokardiyal hasar belirtileri görüldü. TASI tedavileri bu bulguları iyileştirdi ve TASI-10 daha etkili oldu. İmmünohistokimya Sham grubunda β-tubulin, IL-1 ve IL-6'nın minimal ekspresyonunu gösterdi, ancak travma grubunda inflamasyonu gösteren önemli bir yukarı regülasyon gösterdi. Her iki TASI tedavisi de bu belirteçleri azalttı ve TASI-10 en büyük azalmayı gösterdi. Gen ekspresyon analizi travmanın pro-apoptotik genleri (Bax, p53) artırdığını ve anti-apoptotik genleri (Sirt-1, Bcl-2) azalttığını ortaya koydu. TASI-1 gen ifadesini kısmen geri kazandırdı, TASI-10 ise dört gende en önemli gelişmeyi sağladı.

Sonuç: TASI tedavisi, özellikle 10 mg dozda, travmanın neden olduğu miyokardiyal hasarı etkili bir şekilde iyileştiriyor ve histolojik, moleküler ve gen ifadesi seviyelerinde iyileşmeler gözlemleniyor. Bu, TASI'nin miyokardiyal koruma için bir terapötik ajan olarak potansiyel taşıyabileceğini düşündürmektedir.

Anahtar kelimeler: Apoptozis, İnflamasyon, Kardiyoprotektif, Tasimelteon, , Travmatik Beyin Hasarı.

INTRODUCTION

Traumatic brain injury (TBI) causes a complicated chain reaction of secondary ailments that go beyond the central nervous system (CNS), making it a major global public health concern[1]. Among these systemic effects, cardiovascular dysfunction, including neurogenic cardiotoxicity, is increasingly recognized as a major contributor to morbidity and mortality following TBI[2]. The pathophysiology of TBI-induced cardiotoxicity involves excessive catecholamine release, oxidative stress, mitochondrial dysfunction, and apoptosis, which collectively impair myocardial function[3]. Despite advances in neurocritical care, there are limited effective therapeutic strategies to mitigate the detrimental cardiac effects associated with TBI. However, the specific role of Tasimelteon (TASI) in preventing TBI-induced cardiotoxicity remains largely unexplored

TBI triggers various inflammatory processes in the acute and chronic phases, creating systemic effects. Proinflammatory cytokines including interleukin-1 (IL-1) and interleukin-6 (IL-6) are released more often in response to post-injury microglial activation and neuronal stress[4]. These cytokines cross the blood-brain barrier into the peripheral circulation and can lead to significant changes in the cardiovascular system[5].

Inflammation following TBI may cause myocardial dysfunction. When the effects of IL-1 and IL-6 directly on cardiomyocytes are evaluated through cellular skeletal components, beta-tubulin appears to play an important role in this process[6]. Cellular signaling pathways triggered by inflammation may alter the structure of beta-tubulin, leading to disruption of the cellular skeleton, decreased contractility of cardiomyocytes, and disruption of intracellular transport mechanisms[7].

BCL2 associated X (Bax) and B-Cell Lymphoma 2 (Bcl-2) are important proteins in the regulation of apoptosis via the mitochondrial pathway. Bax is a pro-apoptotic protein that promotes cytochrome c release by increasing mitochondrial membrane permeability. Bcl-2, on the other hand, suppresses the activity of Bax by showing anti-apoptotic properties[8]. Changes in the Bax/Bcl-2 balance in cardiac tissue after TBI may increase myocardial cell loss. An increased Bax/Bcl-2 ratio can be considered as an indicator of cardiotoxicity[9].

An essential transcription factor for controlling the cell cycle and initiating apoptosis is p53. Oxidative stress and DNA damage after TBI may increase p53 expression and cause activation of the apoptotic pathway[10]. It has been shown that p53 accelerates cellular death by directly increasing Bax expression. Therefore, increased p53 activity after TBI may trigger programmed cell death in cardiac cells, deepening myocardial damage[11].

The NAD+-dependent deacetylase Sirtuin-1 (SIRT-1) is essential for maintaining energy balance and the physiological stress response. Due to its anti-apoptotic and anti-inflammatory properties, SIRT-1 is known to protect cardiac cells against oxidative stress. In addition, SIRT-1 can suppress the apoptotic process by deacetylating p53[12]. The decrease in SIRT-1 levels after TBI may lead to increased p53 activity and disruption of the Bax/Bcl-2 balance. Therefore, the regulation of SIRT1 may be considered as a therapeutic target for the prevention of cardiotoxicity after TBI[13].

TASI, a selective agonist of the melatonin receptor, has shown neuroprotective qualities by reducing inflammation and oxidative stress. Melatonin and its agonists reduce oxidative stress by neutralizing free radicals. TASI offers some advantages over other melatonin agonists. TASI acts as a potent and selective agonist at MT_1 and MT_2 receptors, making it more specific than non-selective melatonin agonists (e.g. ramelteon). It also differs from other melatonin agonists by its long half-life and low drug interaction, with minimal binding to serotonin and dopamine receptors[14]. A recent study examined the impact of TASI on rats with TBI. The researchers discovered that TASI therapy reduced the oxidative stress index and total oxidant status while increasing total antioxidant status[15]. Melatonin and its analogs have been shown to modulate apoptotic and inflammatory pathways, suggesting their potential role in mitigating TBI-induced systemic damage, including cardiac injury[16]. However, the specific role of TASI in preventing TBI-induced cardiotoxicity remains largely unexplored.

In light of these molecular connections, the current work looks at how TASI may protect against TBI-induced cardiotoxicity by analyzing its effects on SIRT1/p53 signaling, beta-tubulin stability, the Bax/Bcl-2 ratio, and the inflammatory mediators IL-1 and IL-6By clarifying these processes, we want to offer fresh perspectives on TASI''s therapeutic potential for treating cardiac issues brought on by TBI.

METHODS

Ethical Standards

By ARRIVE 2.0 guidelines (Animal Research: Reporting In Vivo Experiments), the protocols used in this study received ethical approval from the Süleyman Demirel. University Local Animal Experiments Ethics Committee (Ref: 09.01.2025/01-435). The research was funded under Project TSG-2024-9515 by Süleyman Demirel University Scientific Research Projects Coordination Unit.

Experimental Groups and Procedures

Rats were obtained from Süleyman Demirel University HÜDAL research laboratory. Each set of thirty-two adult male Wistar albino rats, weighing between 300 and 350 g, was kept

apart from the others in standard Euro-type 4 cages. The rats were kept in a 12-hour light/12-hour dark cycle at 23 °C and 55% humidity. They were given unlimited access to water and regular commercial feed. The following is how the four experimental groups were created:

Group I (Sham): Under anesthesia, rats underwent an incision without inducing trauma. Subsequently, 0.5-1 ml of saline (SF) was administered orally via gavage. The rats were put to death under anesthetic after a day, and tissue samples were taken.

Group II (Trauma): Under anesthesia, rats underwent an incision followed by induced trauma[17]. Subsequently, 0.5-1 ml of saline (SF) was administered orally via gavage. The rats were put to death under anesthetic after a day, and tissue samples were taken.

Group III (Trauma + TASI 1 mg/kg): Under anesthesia, rats underwent an incision followed by induced trauma. Subsequently, 1 mg/kg Tasimelteon was administered orally via gavage. The rats were put to death under anesthetic after a day, and tissue samples were taken[18].

Group IV (Trauma + TASI 10 mg/kg): Under anesthesia, rats underwent an incision followed by induced trauma. Subsequently, 10 mg/kg TASI was administered orally via gavage.

A midline scalp incision was made to expose the skull for trauma induction. Head trauma was induced using the Marmarou impact acceleration model, where a 50 g metal weight was dropped from 80 cm onto a stainless steel disc fixed to the skull, generating a force of 0.2 N. This model reliably produces diffuse axonal injury and systemic inflammatory responses. 17.10 mg/kg xylazine and 90 mg/kg ketamine were used to anesthetize the rats in order to induce trauma and sacrification. Heart tissues were obtained after surgical exsanguination through the inferior vena cava. While the remaining tissues were kept at -80 °C for genetic research, half of the tissues were preserved in formaldehyde for histopathological and immunohistochemical examination. Hematoxylin and eosin staining was used in the histopathological examination to evaluate degenerative necrosis, inflammatory infiltrations, hemorrhage, and hyperemia. β-tubulin, IL-1, and IL-6 levels were examined by immunohistochemistry, and the mRNA expression of the Sirt-1, p53, Bax, and Bcl-2 genes was assessed.

Histopathological Method

Necropsy was used to obtain heart samples, which were then stored in a 10% neutral formalin solution. A fully automated tissue processing machine was subsequently used to embed the heart samples in paraffin wax after regular tissue processing. A fully automated rotary microtome was then used to cut pieces of the paraffin blocks that were 5 µm thick. Hematoxylin-eosin (HE) staining, cover sliding, and light microscopy examination were performed on these sections.

An ordinal grading system was used to semi-quantitatively score the histological lesions in the hearts. Scoring for hyperemia, hemorrhage, inflammatory cell infiltration and degenerative necrotic changes in myocardial cells is shown in Table 1.

Table I: Histopathological Scoring Criteria

Score	Hyperemia	Hemorrhage	Inflammation	Necrosis
0	Absent	Absent	Absent	Absent
1	Mild	Focal	Mild infiltrate	<10% cells
2	Moderate	Multifocal	Moderate infiltrate	10–30% cells
3	Severe	Diffuse	Severe infiltrate	>30% cells

Immunohistochemical examination

Following the manufacturer's directions, three slice series from paraffin blocks were placed on slides coated with poly-L-lysine and stained using the streptavidin-biotin method for immunohistochemical examination. The primary antibodies, which were all diluted to 1/100, were β-tubulin (ab179511), IL-1β (ab283818), and IL-6R (ab300581) Abcam (Cambridge, UK). The streptavidin-alkaline phosphatase conjugate and a biotinylated secondary antibody were added after the primary antibody had been incubated for 60 minutes. The chromogen was diaminobenzidine (DAB), and the secondary antibody was the Mouse and Rabbit Specific HRP/DAB IHC Detection Kit (ab236466). Antigen dilution solution was employed as a negative control. Evaluations were conducted by a pathologist who was blinded. ImageJ 1.48 was used to compute the proportion of positive IHC cells at 40× magnification. Imaging was done using an Olympus CX41 microscope and Cell Sens software (Olympus Corporation, Tokyo, Japan).

Reverse transcription-polymerase chain reaction (RT-qPCR)

To extract RNA from homogenized heart tissues, GeneAll Biotechnology (Korea) used the GeneAll RiboEx™ Kit. To measure the amount and quality of RNA, a BioSpec-nano nanodrop (Shimadzu, Japan) was used. The A.B.T.™ cDNA Synthesis Kit (Atlas Biotechnology, Turkey) and 1 µg of RNA were used for the cDNA synthesis. NCBI mRNA sequences served as the basis for the construction of primers (Table 1). ACTB served as the housekeeping gene for the qPCR analysis of gene expression levels utilizing a Biorad CFX96 system (USA) and 2X SYBR Green Master Mix (Nepenthe, Turkey). As directed by the manufacturer, 20 µl of reactions were made and conducted three times. Melting curve analysis verified the accuracy of the results, and normalization was done using the 2-ΔΔCt method.

Table II: Primary sequences, product size and accession numbers of genes

Genes	Primary sequence	product size	accession number
ACTB (HouseKeeping)	F: CCCGCGAGTACAACCTTCT R: AACACAGCCTGGATGGCT AC	481 bp	NM_031144.3
SIRT1	F: GGTAGTTCCTCGGTGTCCT R: ACCCAATAACAATGAGGAG GTC	152 bp	NM_00141495 9.1
P53	F: CTCCTCTCCCCAGCAAAAG R: CCTGCTGTCTCCTGACTCC T	151 bp	NM_00142999 6.1
BAX	F. CACGTCTGCGGGGAGTCA C R: TAGAAAAGGGCAACCACC CG	419 bp	NM_017059.2
BCL 2	F: CATCTCATGCCAAGGGGG AA R: TATCCCACTCGTAGCCCCT C	284 bp	NM_016993.2

F: Forward, R: Reverse, ACTB: Actin beta, SIRT1: Sirtuin 1, BAX: BCL2 Associated X, BCL 2: B-cell lymphoma 2

Statistical Analysis

Using SPSS, the Shapiro-Wilk test was used to evaluate the data distribution. Since there were fewer than 30 samples in each group and the variances were different, the nonparametric statistical approach one way-ANOVA (posthoc Tukey) was chosen for the data evaluation. Group mean + SD was utilized to express the data, and the appropriate analysis method was used based on the type of data. For all analyses, a value of $p<0.05$ was deemed significant.

RESULTS

Histopathology Findings

The heart tissue of the Sham group showed no pathological alterations upon microscopic inspection. Cardiomyocytes in these 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 the heart, the trauma group showed severe hyperemia (score 3), hemorrhage (score 3), and necrosis (score 2). TASI-1 reduced hyperemia to score 1 and necrosis to score 1, while TASI-10 restored all parameters to almost normal (Figure 1).

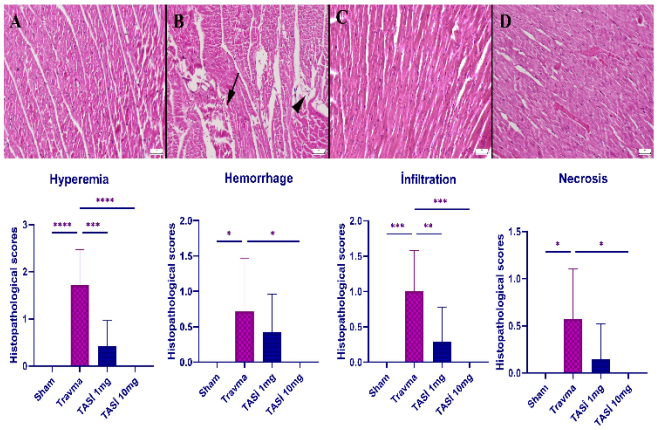

Figure 1. Histopathological findings in heart tissue

Heart histopathology figures that are representative of the groups. (A) Normal histology of myocardial tissue in the Sham group; (B) severe edema (arrowhead) and bleeding (arrow) in the TRAV group; (C) notable improvement in the TRAV+TASI-1 mg group; and (D) nearly normal histology of myocardium in the TRAV+TASI 10 mg group, HE, scale bars=50µm. *p < 0.05, ***p< 0.001, ****p<0.0001

Immunohistochemical examination

Immunohistochemical analyses revealed that the Sham group had extremely little or no expression of β-tubulin, IL-1, and IL-6. On the other hand, β-tubulin, IL-1, and IL-6 expression levels were moderately to significantly higher in the TRAV group's cardiac cells. These pathological outcomes decreased after receiving TASI treatment (Figs. 2.1, 2.2, and 2.3).

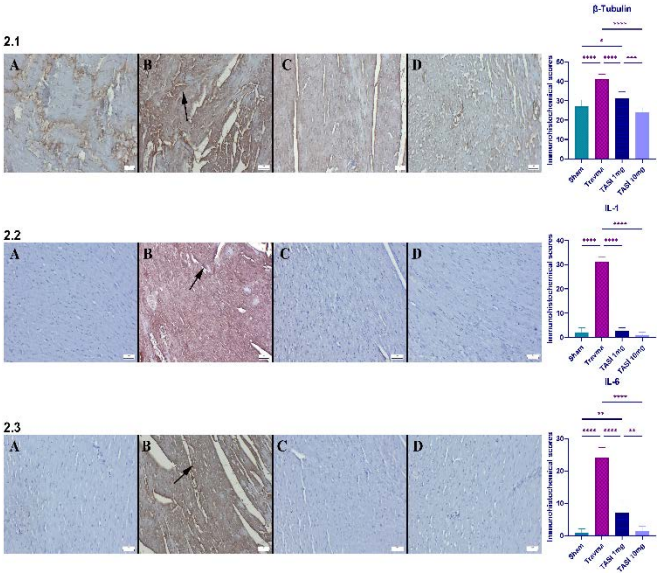

Figure 2. Immunohistochemically β-tubulin, IL-1 and IL-6 expressions of hearts tissue

2.1 Heart β-tubulin expressions were compared between the groups using immunohistochemistry. (A) The Sham group's moderate expression. (B) TRAV group cardiac cells with elevated expressions (arrows). (C) The TRAV+TASI 1 mg group showed decreased expression. (D) Streptavidin biotin peroxidase technique, TRAV+TASI 10 mg group, nearly normal expression, scale bars = 50µm. *p < 0.05, ***p< 0.001, ****p<0.0001

2.2 Heart IL-1 expressions in each group. (A) The Sham group's negative expression. (B) TRAV group cardiac cells with elevated expressions (arrows). (C) Expression was significantly lower in the TRAV+TASI 1 mg group. (D) Streptavidin biotin peroxidase technique, negative expression in TRAV+TASI 10 mg group, scale bars = 50µm. ****p<0.0001

2.3 IL-6 expression in the hearts of the groups, as determined by immunohistochemistry. (A)

The Sham group's negative expression. (B) TRAV group cardiac cells with elevated expressions (arrows). (C) Expression was significantly lower in the TRAV+TASI 1 mg group. (D) Streptavidin biotin peroxidase technique, negative expression in TRAV+TASI 10 mg group, scale bars = 50µm. ** p<0.01, ****p<0.0001

Gene expression analysis results

The expression levels of antiapoptotic Sirt-1 and Bcl-2 genes significantly decreased (p<0.001 for all), whereas the expression levels of apoptotic-related genes Bax and p53 significantly increased in the trauma group relative to the Sham group. Significant improvements in Bax, p53, Sirt-1, and Bcl-2 were found in the TASI 1 mg dosage group as compared to the trauma group (p<0.001, p<0.001, p=0.034, and p=0.029, respectively). All of these gene expression levels showed the greatest improvement at the TASI 10 mg dose (p<0.001 for all) (Figure 3).

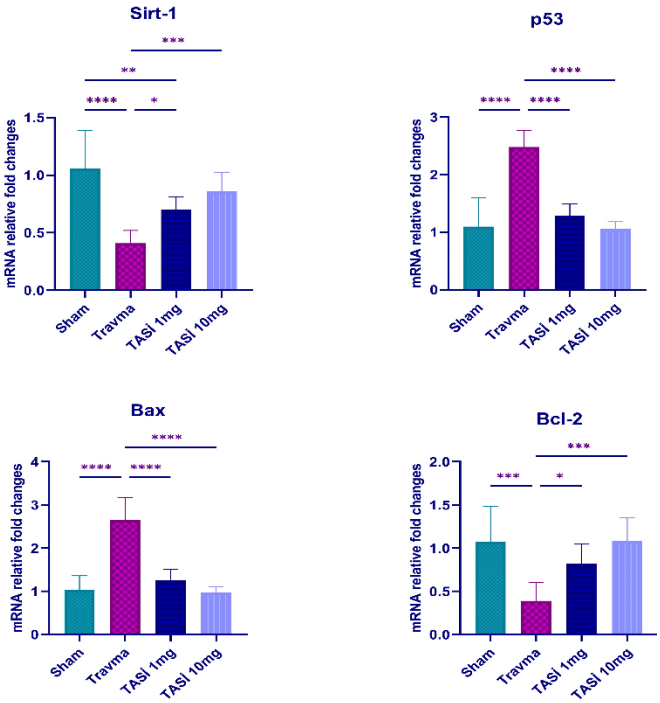

Figure 3. Genes in heart tissue shown by an mRNA relative fold change graph

*SIRT1: Sirtuin 1, BAX: BCL2 Associated X, BCL 2: B-cell lymphoma 2. *p < 0.05, ***p< 0.001, ****p<0.0001*

DISCUSSION

Traumatic brain injury (TBI) is known to precipitate secondary myocardial injury, characterized by structural disruptions, inflammatory responses, and apoptotic alterations[2]. Our study corroborates these findings and further demonstrates the therapeutic potential of TASI-1 and TASI-10 in mitigating such cardiac damage.

Histopathological analyses in our study revealed significant myocardial alterations in the TRAV group, including hyperemia, hemorrhage, and disruption of the cross-striated banding pattern of cardiomyocytes. These results are in line with earlier studies showing that structural damage to the heart might result from TBI. For example, a research by Cuisinier et al. found that TBI may cause cardiac dysfunction, possibly via mechanisms such systemic inflammatory reactions and autonomic dysregulation[19]. Similarly, research by Hüttemann et al. observed left ventricular dysfunction in patients with severe brain injury, suggesting a link between neurological trauma and myocardial impairment[20].

Immunohistochemical assessments in our study revealed elevated expressions of β-tubulin, IL-1, and IL-6 in the TRAV group compared to the Sham group. These findings are consistent with existing literature indicating that traumatic brain injury (TBI) induces a robust inflammatory response, characterized by increased levels of pro-inflammatory cytokines such as IL-1 and IL-6. For instance, a study by Woodcock and Morganti-Kossmann highlighted the pivotal role of the IL-1 family in mediating inflammatory responses both centrally and peripherally following TBI[21]. Similarly, research by Kumar and Loane emphasized that cytokine-mediated inflammation significantly contributes to secondary pathology after TBI, affecting both central and peripheral tissues[22]. These studies support our observations of heightened inflammatory marker expression in

myocardial tissue post-TBI, suggesting a systemic inflammatory response that may contribute to myocardial injury.

Gene expression analyses revealed an upregulation of pro-apoptotic genes Bax and p53, alongside a downregulation of anti-apoptotic genes Sirt-1 and Bcl-2 in the TRAV group. This apoptotic shift aligns with findings from previous studies on traumatic brain injury (TBI), which have demonstrated that neuronal injury can trigger systemic apoptotic signaling pathways affecting peripheral organs, including the heart[23].

The pro-apoptotic protein Bax plays a crucial role in mitochondrial-mediated apoptosis, and its increased expression has been linked to enhanced cell death in various models of brain and cardiac injury[24]. Similarly, p53, a well-known tumor suppressor, functions as a central regulator of apoptosis, particularly in response to cellular stress and DNA damage, and its upregulation has been implicated in TBI-induced neuronal and cardiac apoptosis[25,26]. Conversely, Bcl-2, an anti-apoptotic protein, counteracts mitochondrial outer membrane permeabilization and is essential for cell survival; its downregulation following TBI has been associated with increased susceptibility to apoptosis in both central and peripheral tissues[27]. Moreover, Sirt-1, a NAD+-dependent deacetylase, is known for its neuroprotective and cardioprotective roles, and its suppression has been correlated with oxidative stress, inflammation, and apoptosis in post-TBI pathology[28,29]. These findings collectively support the hypothesis that TBI induces a systemic apoptotic response that extends beyond the central nervous system, potentially contributing to secondary organ damage through interconnected molecular pathways.

Earlier research demonstrated that caspase inhibitors and anti-inflammatory agents could reduce myocardial apoptosis and improve cardiac outcomes post-TBI[30]. However, these interventions often lacked specificity and exhibited limited cardioprotective effects. In contrast, the present study shows that TASI-1 and TASI-10 compounds specifically target apoptotic regulators, resulting in notable amelioration of pathological changes. Importantly, TASI-10 demonstrated superior efficacy compared to TASI-1, highlighting the potential of this compound for more effective cardioprotection[15,31].

CONCLUSION

In conclusion, our study reinforces the association between TBI and secondary myocardial injury, highlighting the role of inflammatory and apoptotic mechanisms. TASI provides cardioprotective benefits by showing a therapeutic effect at doses of 10 mg/kg. Future research should focus on elucidating the precise molecular mechanisms underlying TASI's effects and exploring its potential clinical applications in managing TBI-induced cardiac injury.

Ethics Committee Approval: By ARRIVE 2.0 guidelines (Animal Research: Reporting In Vivo Experiments), the protocols used in this study received ethical approval from the Suleyman Demirel University Local Animal Experiments Ethics Committee (Ref: 09.01.2025/01-435).

Conflict of Interest: The authors declared no conflicts of interest.

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REFERENCES

1. Maas AIR, Menon DK, Adelson PD, et al. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. Lancet Neurol. 2017;16(12):987-1048.

2. Krishnamoorthy V, Mackensen GB, Gibbons EF, Vavilala MS. Cardiac dysfunction after neurologic injury: what do we know and where are we going? Chest. 2016;149(5):1325-31.

3. Fesharaki-Zadeh A. Oxidative stress in traumatic brain injury. Int J Mol Sci. 2022;23(21):13000.

4. Liu X, Zhang L, Cao Y, et al. Neuroinflammation of traumatic brain injury: Roles of extracellular vesicles. Front Immunol. 2023;13:1088827.

5. Liberale L, Ministrini S, Carbone F, Camici GG, Montecucco F. Cytokines as therapeutic targets for

cardio-and cerebrovascular diseases. Basic Res Cardiol. 2021;116(1):23.

6. Maass DL, White J, Horton JW. IL-1β and IL-6 act synergistically with TNF-α to alter cardiac contractile function after burn trauma. Shock. 2002;18(4):360-6.

7. Caporizzo MA, Prosser BL. The microtubule cytoskeleton in cardiac mechanics and heart failure. Nat Rev Cardiol. 2022;19(6):364-78.

8. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol cell Biol. 2014;15(1):49-63.

9. Özmen Ö, Aşçı H, Savran M, Şahin M, Ozmen O. Amantadine preserve VCAM immunoexpression levels in cardiac injury induced by brain trauma with its anti-inflammatory action and protective effect on the mitochondrial membrane. Mehmet Akif Ersoy Univ J Heal Sci Inst. 2024;12(3):15-23.

10. Ismail H, Shakkour Z, Tabet M, et al. Traumatic brain injury: oxidative stress and novel anti-oxidants such as mitoquinone and edaravone. Antioxidants. 2020;9(10):943.

11. Rodkin S, Nwosu C, Raevskaya M, et al. The Role of Hydrogen Sulfide in the Localization and Expression of p53 and Cell Death in the Nervous Tissue in Traumatic Brain Injury and Axotomy. Int J Mol Sci. 2023;24(21):15708.

12. Vaziri H, Dessain SK, Eaton EN, et al. hSIR2SIRT1 functions as an NAD-dependent p53 deacetylase. Cell. 2001;107(2):149-59.

13. Chen M, Liu J, Wu W, et al. SIRT1 restores mitochondrial structure and function in rats by activating SIRT3 after cerebral ischemia/reperfusion injury. Cell Biol Toxicol. 2024;40(1):31.

14. Cecon E, Oishi A, Jockers R. Melatonin receptors: molecular pharmacology and signalling in the context of system bias. Br J Pharmacol. 2018;175(16):3263-80.

15. Özden ES, Özcan MS, Savran M, et al. Effects of Tasimelteon Treatment on Traumatic Brain Injury Through NRF-2/HO-1 and RIPK1/RIPK3/MLKL Pathways in Rats. Mol Neurobiol. Published online 2025:1-10.

16. Sieminski M, Reimus M, Kałas M, Stępniewska E. Antioxidant and Anti-Inflammatory Properties of Melatonin in Secondary Traumatic Brain Injury. Antioxidants. 2024;14(1):25.

17. Marmarou A, Foda MAAE, Van Den Brink W, et al. A new model of diffuse brain injury in rats: Part I: Pathophysiology and biomechanics. J Neurosurg. 1994;80(2):291-300.

18. Hardeland R. Investigational melatonin receptor agonists. Expert Opin Investig Drugs. 2010;19(6):747-64.

19. Cuisinier A, Maufrais C, Payen JF, et al. Myocardial function at the early phase of traumatic brain injury: a prospective controlled study. Scand J Trauma Resusc Emerg Med. 2016;24:1-7.

20. Hasanin A, Zakaria D, Allam A. Cardiac injury in severe head trauma: a review of literature. J Neurol Neuromedicine. 2016;1(8).

21. Woodcock T, Morganti-Kossmann MC. The role of markers of inflammation in traumatic brain injury. Front Neurol. 2013;4:18.

22. Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav Immun. 2012;26(8):1191-201.

23. Yang Q, Zhou Y, Sun Y, et al. Will sirtuins be promising therapeutic targets for TBI and associated neurodegenerative diseases? Front Neurosci. 2020;14:791.

24. Borutaite V, Toleikis A, Brown GC. In the eye of the storm: mitochondrial damage during heart and brain ischaemia. FEBS J. 2013;280(20):4999-5014.

25. Plesnila N, Von Baumgarten L, Retiounskaia M, et al. Delayed neuronal death after brain trauma involves p53-dependent inhibition of NF-κB transcriptional activity. Cell Death Differ. 2007;14(8):1529-41.

26. Sabet N, Soltani Z, Khaksari M. Multipotential and systemic effects of traumatic brain injury. J Neuroimmunol. 2021;357:577619.

27. Emir M, Ozisik K, Cagli K, et al. Effect of erythropoietin on bcl-2 gene expression in rat cardiac myocytes after traumatic brain injury. In: Transplantation Proceedings. Vol 36. Elsevier; 2004:2935-8.

28. Wei G, Wang J, Wu Y, et al. Sirtuin 1 alleviates neuroinflammation-induced apoptosis after traumatic brain injury. J Cell Mol Med. 2021;25(9):4478-86.

29. Wei C, Wang J, Yu J, et al. Therapy of traumatic brain injury by modern agents and traditional Chinese medicine. Chin Med. 2023;18(1):25.

30. Jarrahi A, Braun M, Ahluwalia M, et al. Revisiting traumatic brain injury: from molecular mechanisms to therapeutic interventions. Biomedicines. 2020;8(10):389.

31. Chen Z, Venkat P, Seyfried D, et al. Brain–heart interaction: cardiac complications after stroke. Circ Res. 2017;121(4):451-68.