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Protective effects of hypoxic conditioning treatment on brain and cardiac tissues following thoracic aorta occlusion

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Abstract:

BACKGROUND: Thoracic aortic ischemia-reperfusion (I/R) injury occurs in clinical scenarios and can lead to damage in organs such as the spinal cord, kidneys, and intestines. Hypoxic postconditioning (HyP) has shown promise in reducing organ I/R injury, suggesting its potential applicability in thoracic aortic I/R injury. However, the pathological damage caused by thoracic aorta occlusion (TAO) to the heart and brain is not yet well understood. This study aims to investigate the protective effects of hypoxic conditioning (HyP) treatment on brain and cardiac tissues following TAO-induced I/R injury.

MATERIALS AND METHODS: Male C57BL/6 mice were used to construct the TAO model by blocking the thoracic aorta for 0.5 or 1 h, followed by 24 h of reperfusion. The mice were divided into five groups: sham, TAO (0.5 h), TAO (0.5 h) +HyP, TAO (1 h), and TAO (1 h) +HyP. Hematoxylin and eosin, Masson, and Sirius red staining were performed to assess morphological changes and collagen deposition in brain and heart tissues. Protein expression assays were conducted to quantify inflammation-related proteins in the serum.

RESULTS: The results showed that TAO caused significant neuronal damage in the hippocampal regions (CA1, CA3, and DG) and myocardial cell damage with collagen deposition. HyP treatment significantly alleviated these damages, particularly with shorter ischemic durations (0.5 h). Specifically, in cardiac tissues, HyP treatment reduced myocardial injury and collagen deposition. In addition, HyP treatment modulated systemic inflammatory responses, as evidenced by the increased expression of anti-inflammatory proteins such as interleukin 13 (IL-13) and the decreased expression of pro-inflammatory proteins such as IL-6, IL-12p70, IL-17, and tumor necrosis factor-α.

CONCLUSION: HyP treatment significantly mitigates brain and cardiac tissue damage caused by TAO, especially with shorter ischemic durations. These findings highlight the potential clinical application of HyP treatment in reducing TAO-induced tissue damage and inflammation, offering a novel therapeutic option for patients with thoracic aortic I/R injury. Future studies should further investigate the mechanisms and optimal implementation protocols of HyP treatment to maximize its clinical value.

Keywords:

Brain tissue, Cardiac tissue, Hypoxic conditioning, Inflammation, Ischemia-reperfusion injury, Thoracic aorta occlusion

Introduction

Thoracic aortic ischemia-reperfusion (I/R) injury primarily occurs in various clinical scenarios, including resuscitative endovascular balloon occlusion of the aorta (REBOA) during severe hemorrhagic events, aneurysm repair, or aortic replacement and coronary artery bypass

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grafting.[1-4] These injury results from the temporary interruption of blood flow followed by its restoration, which can lead to ischemic damage across multiple organ systems. The consequences of thoracic aortic I/R injury are profound, potentially affecting the spinal cord, kidneys, and intestines, as well as the heart and brain,[5-8] leading to both functional impairments and structural damage. Recognizing the different clinical contexts in which this injury can occur is essential for developing effective strategies to mitigate its adverse effects and enhance patient outcomes.

The heart and brain are of particular interest due to their essential roles in survival – maintaining circulatory stability and regulating vital functions, respectively.[9,10] Their high metabolic demands render them especially vulnerable to disruptions in blood supply, which can result in severe and potentially irreversible damage.[11] This susceptibility highlights the urgent need for targeted research to understand and mitigate the impacts of I/R injury on these critical organs.

To address the challenges posed by thoracic aorta I/R injury, researchers have been exploring various therapeutic strategies aimed at reducing tissue damage and improving outcomes.[3,12-15] One such approach that has shown promise is hypoxic postconditioning (HyP), a technique that involves subjecting tissues to cycles of hypoxia and reoxygenation during the reperfusion phase.[16-18] By modulating oxidative stress, inflammation, and cell death pathways, HyP has the potential to protect tissues from the harmful effects of ischemia/reperfusion injury.[16,19] In clinical trials, intermittent hypoxic training, a related technique, has been found to improve mood, health, and metabolism in patients with multiple sclerosis;[20] enhance cognitive function in patients with mild cognitive impairment;[21] and promote the recovery of hand function after spinal cord injury.[22] These findings highlight the broad potential of hypoxia-based therapies in various clinical contexts. Our preliminary research has demonstrated that HyP can significantly reduce ischemic reperfusion injuries in the abdominal aorta occlusion mice mode,[23] suggesting its applicability in thoracic scenarios. These indicate the potential clinical value of HyP in treating thoracic aorta I/R injury.

In this study, we aimed to investigate the efficacy of HyP in mitigating thoracic aorta ischemia/reperfusion injury in mouse models. By utilizing a thoracic aorta occlusion (TAO) model and implementing HyP treatment protocols, we first explored the impact of varying durations of TAO on the pathology of heart and brain tissues to establish a baseline of ischemic damage. Subsequently, we implemented HyP treatment protocols to determine whether this approach could reverse the tissue damage induced by TAO. This research aims to contribute to the development of effective therapeutic strategies for reducing tissue damage and improving outcomes in patients experiencing thoracic aortic I/R injury.

Materials and Methods

Animals

Male C57BL/6 mice (8 weeks old) were used in this experiment, which were purchased from SPF (Beijing) Biotechnology Co., Ltd. All animals were housed individually in a room with lighting (12-h light–dark cycle) and controlled temperature (22 ± 2°C), and had access to water and food freely.

Animals model and experiment design

In this study, the TAO model was used to investigate thoracic aorta ischemia/reperfusion injury in heart and brain tissues. The mice were induced anesthesia with 5% enflurane and maintained anesthesia with 1.5% enflurane, 30% O_2, and 68.5% N_2O, and restrained in a supine position. Subsequently, the neck and chest were shaved and disinfection and the ribs were cut open to expose the aorta. The mice's body temperature was maintained at 37°C with a heating pad. All mice were randomly divided into five groups (n = 5 per group): sham, TAO (0.5 h), TAO (0.5 h) +HyP, TAO (1 h), and TAO (1 h) +HyP. In the sham group, the mice only performed with a laparotomy. In the TAO groups, the thoracic aorta 0.5 cm below the subclavian artery of the mice was sutured with a purse-string suture using 7-Prolene thread for 0.5 h or 1 h, and then followed by 24 h reperfusion. After all procedures were completed, the mice were subjected to blood collection, then sacrificed and sampled brain and heart tissue samples. Experimental design is shown in Figure 1.

Hypoxic postcondition treatment

After successfully constructing the TAO model for 0.5 h or 1 h, the mice in TAO + HyO groups were treated with hypoxic postconditioning. The process of hypoxic postconditioning was performed as described in a previous study, with slight modification.[23] Briefly, 10 min after establishing the TAO model, the mice were placed in a hypoxic chamber, where the oxygen concentration was gradually reduced to 5% over a period of 20 min. This hypoxic treatment was maintained for a total of 20 min, ensuring a consistent and controlled hypoxic environment. The relative humidity within the chamber was maintained at approximately 50% to prevent dehydration and to mimic the natural breathing conditions of the mice. Oxygen levels were continuously monitored using a calibrated oxygen sensor to ensure the accuracy and stability of the hypoxic environment.

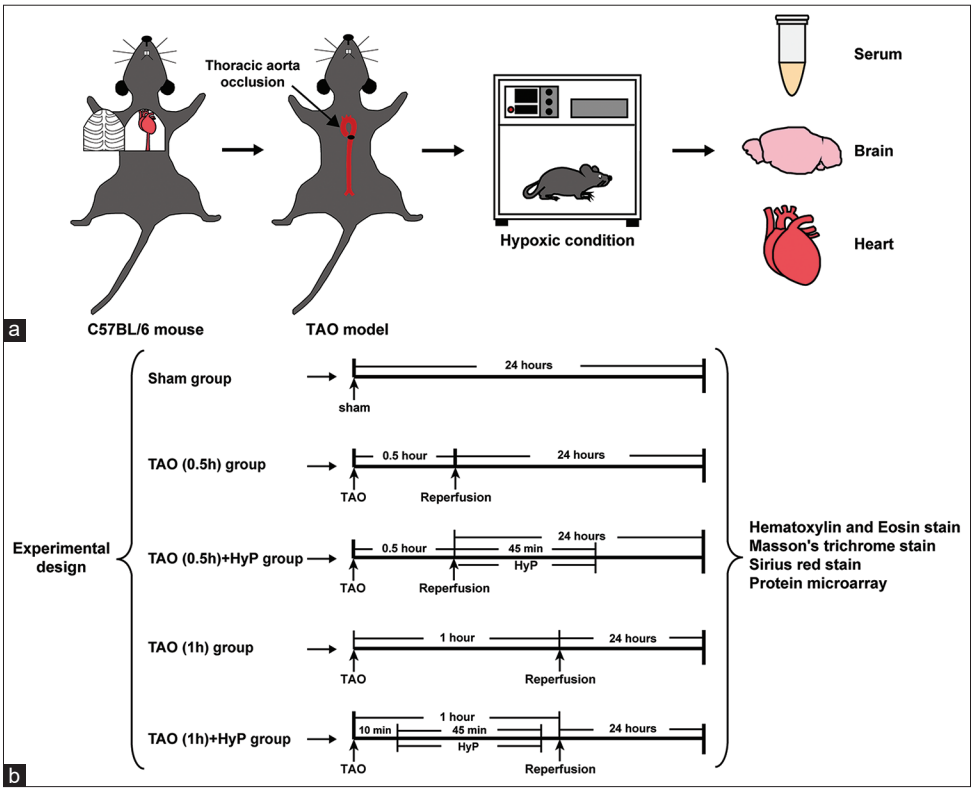

Figure 1: Schematic presentation of this experimental design. (a) Male C57BL/6 mice were used to construct the thoracic aorta occlusion model by blocking the thoracic aorta 0.5 cm below the subclavian artery with a suture for 0.5 h or 1 h. Then treat under hypoxia condition, and extracted the tissues of serum, brain, and heart for test. (b) The timeline of the experiment process of all groups. TAO: Thoracic aorta occlusion

Hematoxylin and eosin staining analysis

The mice were anesthetized and transcardially perfused with cold normal saline, and then heart and brain tissues were taken and fixed with 4% paraformaldehyde fixative solution, embedded in paraffin, and sectioned to a 4.5-μm thickness slices. Subsequently, hematoxylin and eosin (H and E) staining was used to observe the morphological changes in the brain and myocardium, as described in the previous study.[23] Briefly, all slices were scanned by an automatic microscopic scanner (Nikon, Japan). Five fields were randomly selected from slices in different groups. The nuclei are appear blue-purple, while the cytoplasm and extracellular matrix are pink. The neurohistological analysis involves counting and quantifying the damaged neuronal cells in the hippocampus at high-power fields of sections. The total myocardial histological score are as follows: slight granule denaturation (←, 1 point), vacuolar degeneration (→, 2 points), infiltration (Δ, 3 points), and necrosis (↑, 4 points).

Sirius red and Masson staining analysis

In this experiment, Sirius Red and Masson staining were used to monitor the status of collagen deposition in the myocardium. Sirius Red staining as described in previous study,[23] collagen fibers appear red or bright red, while others appear yellow. To perform Masson staining on paraffin-embedded tissue of the heart, the sections were incubated in a series of Masson solutions (Servicebio, China) at room temperature. To increase the birefringence of the collagen fibers, the sections were rinsed and differentiated with 1% acetic acid and dehydrated with absolute ethanol. Subsequently, slices were transparently treated with xylene for 5 min and sealed with neutral resin. Collagen fibers appear blue, while muscle fibers, cellulose, and red blood cells appear red. Briefly, all slices were scanned by an automatic microscopic scanner (Nikon, Japan). Five fields were randomly selected from slices in different groups. The myocardial damage was quantified in the collagen area and fibrosis area by ImageJ software (Bethesda, USA).

Protein expression assay

The Quantibody® Mouse Inflammation Array 1 Glass Chip (RayBiotech, Norcross, GA) was used to determine the expression of inflammation relation proteins in the serum. Protein expression assay as described in the previous study.[24] The Quantibody Mouse Inflammation Array 1 was used to quantify 40 cytokines. The binding of each protein on the membrane was revealed by autoradiography, and the signals were scanned using the laser scanner (Innopsys, France). Finally, RayBio QAM-INF-1 analysis tool (Ray Biotech, Inc.) was used for data analysis.

Statistical analysis

Statistical analyses and histograms generated were performed with GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). All results are expressed as mean ± standard error of the mean. Shapiro–Wilk test was applied for the evaluation of normality for each set of data compared. For normally distributed populations of data points, a two-tailed unpaired Student's *t*-test was used for comparisons between the two groups. The ANOVA analysis followed by Tukey's honestly significant different test was used to analyze the differences among three or more groups. For data that failed the normality test, a Mann–Whitney test (2 groups) or a Kruskal–Wallis test with a Dunn posttest (>2 groups). $P < 0.05$ was considered statistically signification. Each figure indicates the sample size and the accompanying *P* value.

Results

Thoracic aorta occlusion-induced pathological damage in hippocampus

To study the effects of TAO on rat brain tissue, H and E staining analysis was conducted. The results showed that TAO caused damage to the neuronal cells, particularly in the hippocampal regions such as CA1, CA3, and DG [Figure 2]. The damaged neuronal cells exhibited nuclear pyknosis, which is characterized by the condensation of the cell nucleus. Compared to the sham group, the damage in the TAO group was more significant, with a *P* value of $P < 0.05$, $n = 5$. This indicates that TAO causes significant damage to brain tissue. However, the extent of neuronal damage did not increase with longer durations of TAO, as there was no significant difference observed between the 0.5 h and 1 h occlusion groups.

Thoracic aorta occlusion-induced pathological damage in heart

To explore the effects of TAO on cardiac tissue, H and E, Masson, and Sirius staining analyses were performed. The results indicated that TAO caused myocardial cell damage and led to collagen deposition [Figure 3]. Figure 3a shows H and E staining, which revealed disorganized myocardial fibers and damaged cardiomyocytes in the TAO group, indicated by myocardial histological score. Although there was no significant difference between the 0.5 h and 1 h occlusion groups, the myocardial damage score in the 1 h group was significantly higher than in the sham group ($P < 0.0001$). Figure 3b displays Masson staining, which is used to detect collagen fibers. The results were consistent with those of Figure 3a, showing a significant increase in collagen deposition in the 1 h TAO group compared to the sham group ($P < 0.0001$). Figure 3c presents Sirius staining, which is also used to visualize collagen fibers. The findings mirrored those observed with H and E and Masson staining, indicating a significant increase in collagen deposition in the 1 h TAO group ($P < 0.0001$).

Thoracic aorta occlusion-induced changes in serum inflammatory protein expression

To understand the impact of TAO on the expression of inflammatory-related proteins in serum, a protein chip assay was conducted. The results showed that TAO caused changes in the expression of serum inflammatory proteins [Figure 4].

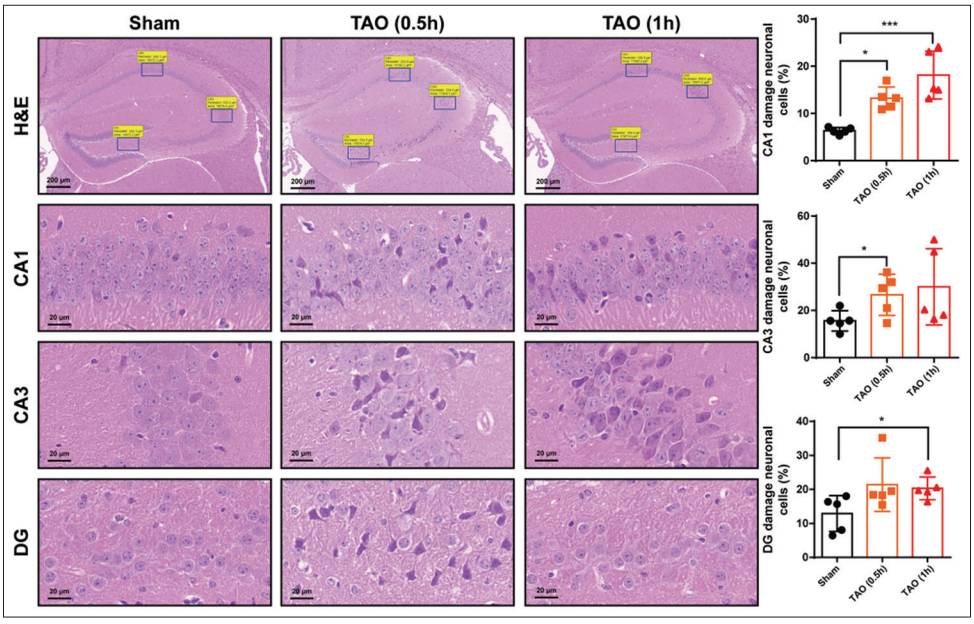

Figure 2: The pathological damage inflicted by thoracic aorta occlusion on brain. Representative images of hematoxylin and eosin staining of brain, the deep staining of CA1, CA3, and DG represented the damage neuronal cells in the hippocampus. Data are presented as mean ± standard error of the mean, versus sham group, $^{*}P < 0.05$, $^{***}P < 0.001$, $n = 5$. The scale bar for the entire hippocampus region is 200 μm and for the local region (CA1, CA3, and DG) is 20 μm

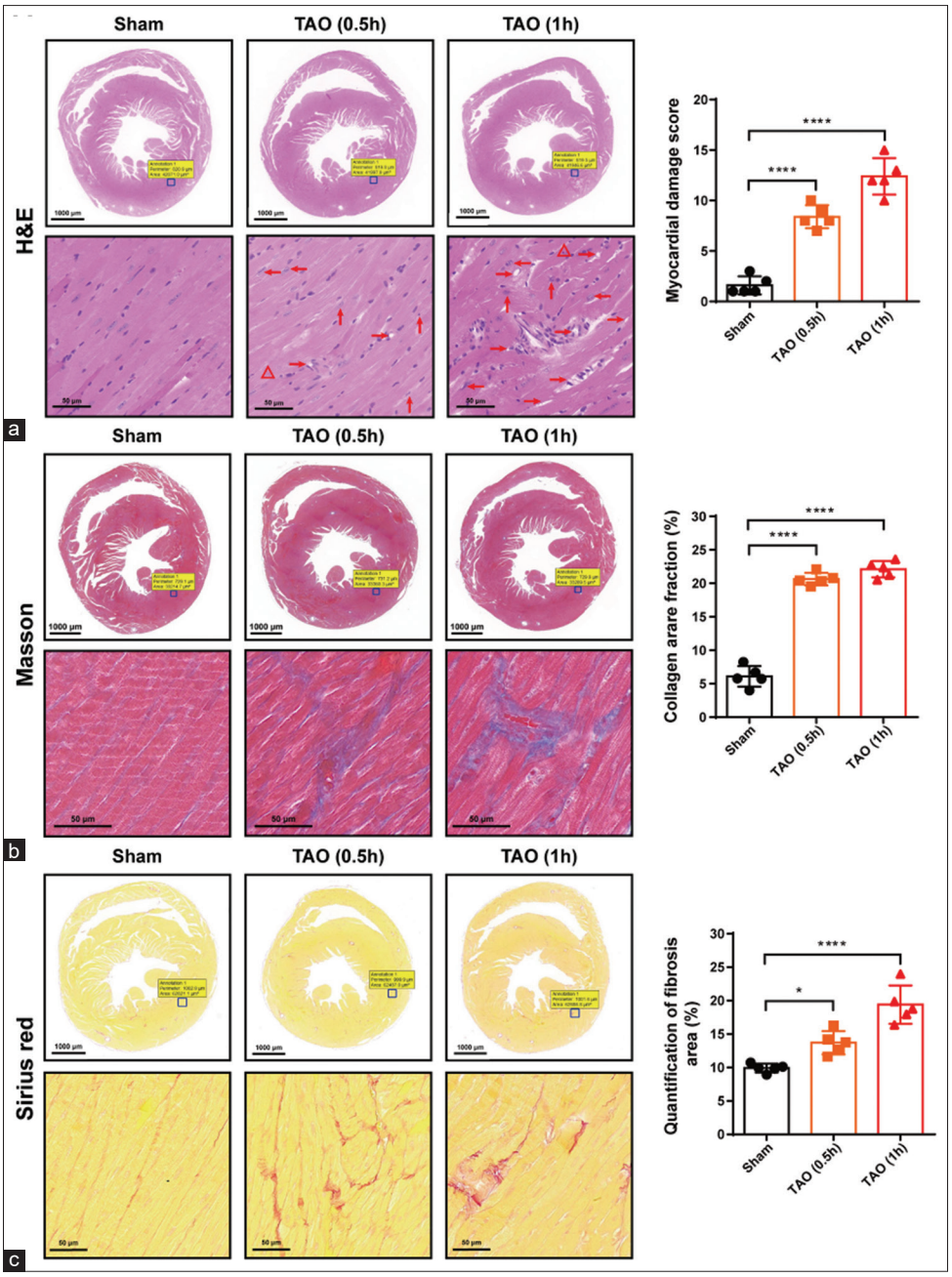

Figure 3: The pathological damage inflicted by thoracic aorta occlusion on heart. (a) Representative images of hematoxylin and eosin staining of myocardium, these arrows and symbols indicated the damage myocardial cells in the myocardium. Data are presented as mean ± standard error of the mean (SEM), versus sham group, ****P < 0.0001, n = 5. (b) Representative images of Masson staining of myocardium, blue is represented collagen deposition. Data are presented as mean ± SEM, versus sham group, ****P < 0.0001, n = 5. (c) Representative images of Sirius staining of myocardium, red is represented collagen deposition. Data are presented as mean ± SEM, versus sham group, *P < 0.05, ****P < 0.0001, n = 5. The scale bar for the entire myocardium region is 1,000 μm and for the local region is 50 μm

Anti-inflammatory proteins such as interleukin 5 (IL-5), IL-6, IL-13, and IL-21 decreased, while pro-inflammatory proteins such as tumor necrosis factor (TNF) TNFRSF1A (RI), Tissue Inhibitor of Metalloproteinases-1 (TIMP-1), CD30 L, and Macrophage Inflammatory Protein-1α (MIP-1α) increased. Compared to the sham group, these differences were statistically significant, with P values ranging from P < 0.05 to ***P < 0.0001. These findings indicate that TAO induces notable changes in the inflammatory response. This alteration in the expression of inflammatory proteins highlights the potential for identifying specific inflammatory markers that could be used in clinical settings for the diagnosis or monitoring of TAO-induced damage.

HyP treatment reduced pathological damage in hippocampus

To evaluate the effect of HyP treatment on TAO-induced brain tissue damage, H and E staining analysis was

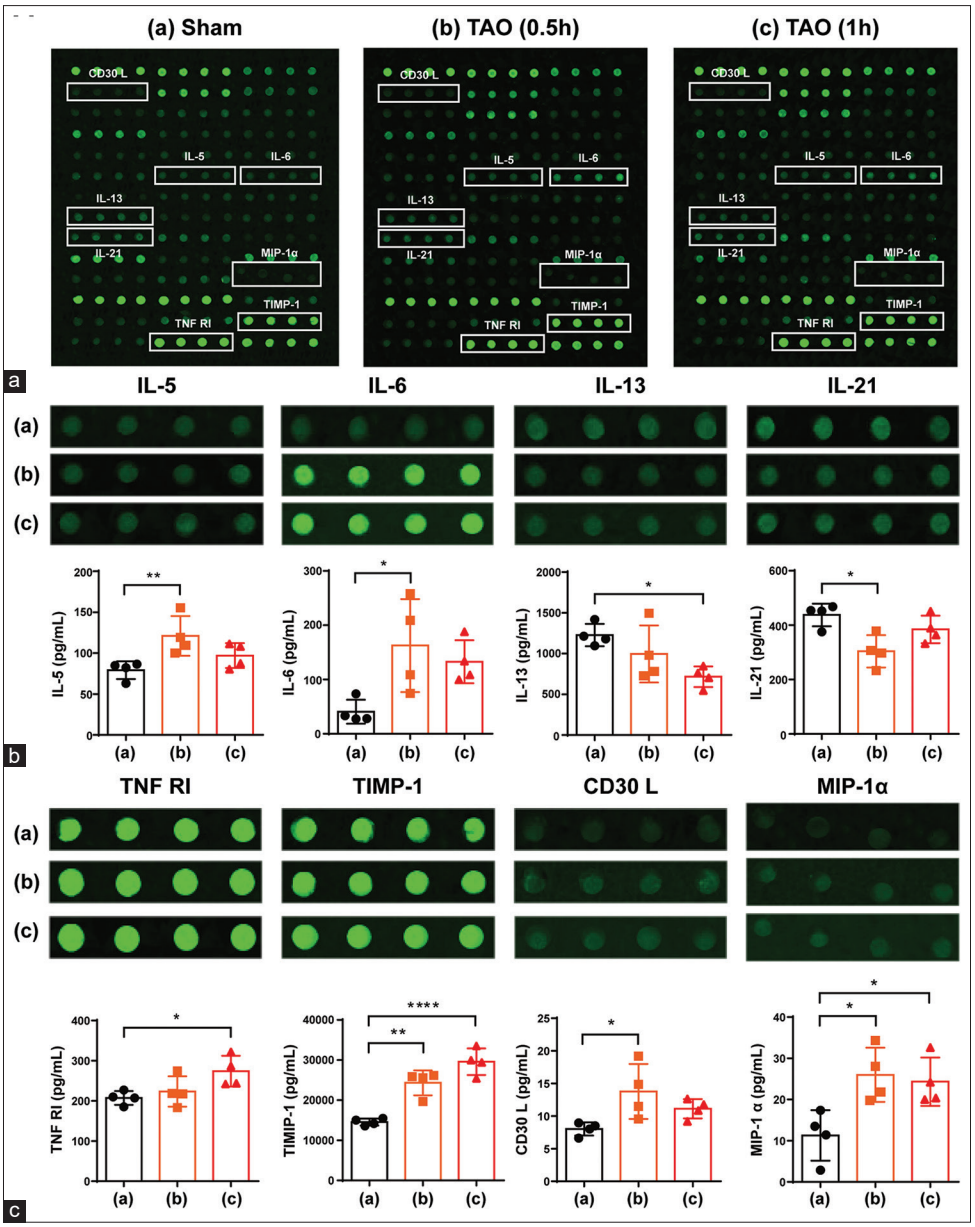

Figure 4: Differential expression of inflammation-related proteins inflicted by thoracic aorta occlusion in the serum. (a) Representative images of the quantitative inflammation array of protein chip detection. (b) Representative images of expressed anti-inflammation proteins: Interleukin-5 (IL-5), IL-6, IL-13, and IL-21. Data are presented as mean ± standard error of the mean (SEM), versus sham group, **P* < 0.05, ***P* < 0.01, *n* = 4. (c) Representative images of expressed pro-inflammation proteins: tumor necrosis factor TNFRSF1A (RI), Tissue Inhibitor of Metalloproteinases-1 (TIMP-1), CD 30 L, and Macrophage Inflammatory Protein-1α (MIP-1α). Data are presented as mean ± SEM, versus sham group, **P* < 0.05, ***P* < 0.01, *****P* < 0.0001, *n* = 4

performed. The results showed that HyP treatment significantly improved the damage caused by TAO to brain tissue [Figure 5]. Compared to the TAO group, neuronal cell damage in the HyP treatment group was reduced, with a *P* value of $P < 0.05$, $n = 5$. Specifically, in the TAO 0.5-h occlusion group, HyP treatment reversed the damage in the hippocampal CA1, CA3, and DG regions [Figure 5a]. However, in the TAO 1-h occlusion group, HyP treatment only reversed the damage in the CA1 region. These observations were made 24 h after reperfusion [Figure 5b]. These findings suggest that the protective effects of HyP treatment are more effective in reversing damage caused by shorter ischemic periods (0.5-h occlusion) and are particularly beneficial in the CA1 region of the hippocampus, even after longer ischemic periods (1-h occlusion).

HyP treatment reduced the pathological damage of cardiac tissue

To study the effect of HyP treatment on TAO-induced cardiac tissue damage, both H and E and Masson's trichrome staining analyses were conducted. The results showed that HyP treatment significantly reduced the

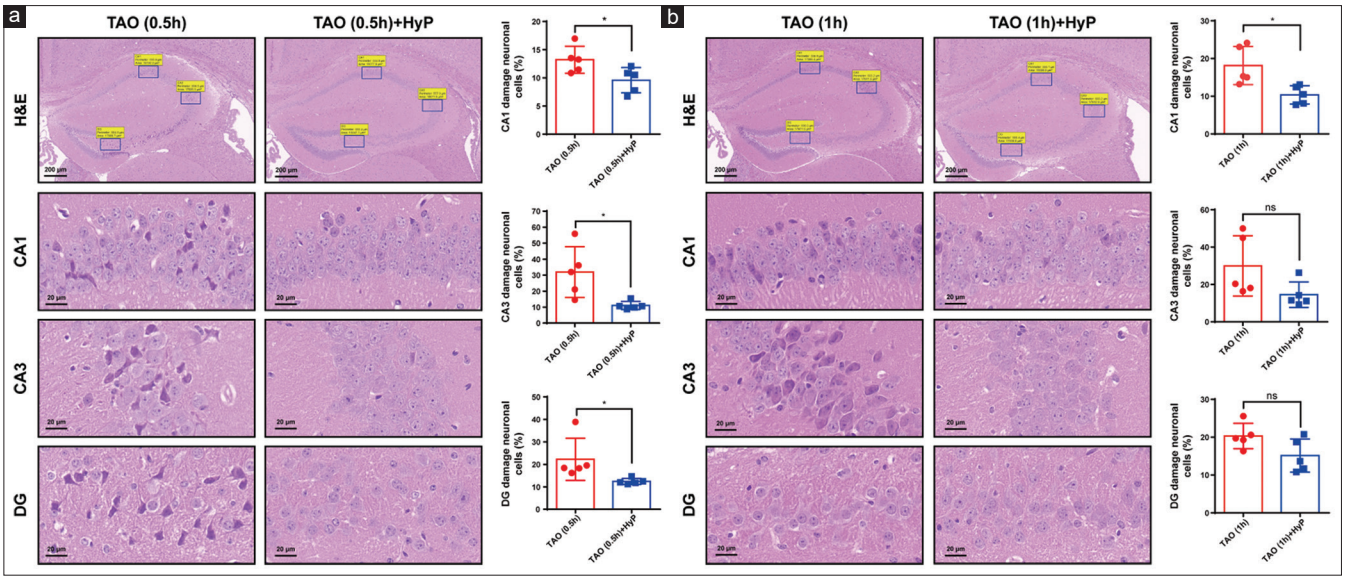

Figure 5: HyP therapy improved the pathological damage inflicted by thoracic aorta occlusion (TAO) on brain. (a) Representative images of hippocampus with hematoxylin and eosin (H and E) staining in the TAO (0.5 h) group and TAO (0.5 h) +HyP group, the deep staining of CA1, CA3, and DG represented the damaged neuronal cells in the hippocampus. Data are presented as mean ± standard error of the mean (SEM), versus TAO (0.5 h) group, **P* < 0.05, *n* = 5. (b) Representative images of hippocampus with H and E staining in TAO (1 h) group and TAO (1 h) + HyP group, the deep staining of CA1, CA3, and Dentate gyrus (DG) represented the damaged neuronal cells in the hippocampus. Data are presented as mean ± SEM, versus TAO (1 h) group, **P* < 0.05, *n* = 5. The scale bar for the entire hippocampus region is 200 μm and for the local region (CA1, CA3, and DG) is 20 μm

damage and collagen deposition caused by TAO in cardiac tissue [Figure 6]. Compared to the TAO group, myocardial damage and collagen deposition in the HyP treatment group were significantly reduced, with *P* values ranging from ***P* < 0.001 to ****P* < 0.0001. This indicates that HyP treatment can protect cardiac tissue from damage caused by TAO.

HyP treatment inhibited the levels of inflammatory factors in serum

To explore the effect of HyP treatment on the expression of inflammatory-related proteins in serum caused by TAO, a protein chip assay was conducted. The results showed that HyP treatment modulated the inflammatory response induced by TAO. Specifically, the levels of several inflammatory factors, including Granulocyte colony stimulating factor (G-CSF), IL-6, IL-12p70, IL-17, TNF-α, interferon gamma, and G-CSF, were significantly reduced in the HyP treatment group compared to the TAO 0.5-h group. In addition, the levels of certain factors such as leptin, IL-13, and IL-21 were elevated in the HyP treatment group [Figure 7].

Similarly, the results showed that HyP treatment modulated the inflammatory response induced by TAO in the 1-h group. Specifically, the levels of several inflammatory factors, including IL-15, TNFRI, TNFRII, C-GSF, TIMP-1, and TCA-3, were significantly reduced in the HyP treatment group compared to the TAO 1-h group. In addition, the level of IL-13 was elevated in the HyP treatment group [Figure 8].

These results indicate that HyP treatment effectively mitigates the inflammatory response triggered by TAO at both 0.5-h and 1-h intervals. The reduction in key inflammatory factors suggests a broad anti-inflammatory effect of HyP.

Discussion

Our study demonstrates that TAO leads to significant damage in remote organs, specifically the heart and brain. HyP treatment significantly alleviated this damage, particularly with shorter ischemic durations. In addition, HyP treatment reduced systemic inflammatory responses. These findings highlight the potential clinical application of HyP treatment in mitigating TAO-induced tissue damage and inflammation.

Thoracic aorta occlusion-induced the tissue injury above the aortic occlusion

In the study of nervous system injuries, multiple studies have demonstrated that TAO leads to spinal cord injury. For instance, Awad *et al*. reviewed the histological findings after aortic cross-clamping in preclinical animal models and found that spinal cord injury is commonly observed following TAO.[25] In addition, research has shown that TAO can alter cerebral blood flow,[26] which may lead to brain tissue damage.[15] Building on these insights, we investigated the effects of TAO on brain tissue. Our study indicates that TAO results in neuronal cell damage in the hippocampal CA1, CA3, and DG regions, characterized by nuclear condensation. This damage reaches its maximum level during the initial occlusion period (30 min), and prolonging the TAO time (60 min) does not further exacerbate the injury. This

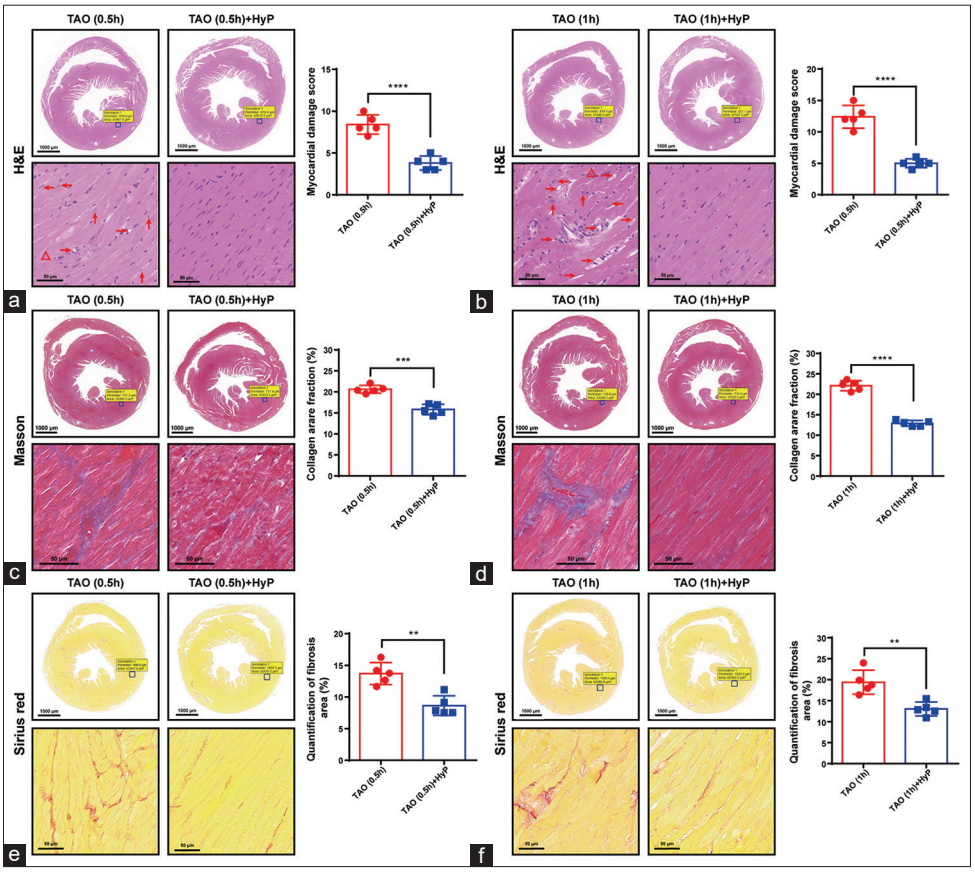

Figure 6: HyP therapy improved the pathological damage inflicted by thoracic aorta occlusion (TAO) on heart. (a and b) Representative images of myocardium with hematoxylin and eosin staining in TAO groups and TAO + HyP groups, these arrows and symbols indicated the damage myocardial cells in the myocardium. Data are presented as mean ± standard error of the mean (SEM), versus TAO group, ****$P < 0.0001$, $n = 5$. (b and c) Representative images of myocardium with Masson staining in TAO groups and TAO + HyP groups, blue is represented collagen deposition. Date are presented as mean ± SEM, versus TAO group, ***$P < 0.001$, ****$P < 0.0001$, $n = 5$. (c) Representative images of the myocardium with Sirius staining in TAO groups and TAO + HyP groups, red is represented collagen deposition. Date are presented as mean ± SEM, versus TAO group, **$P < 0.01$, $n = 5$. The scale bar for the entire myocardium region is 1,000 μm and for the local region is 50 μm

suggests that the initial period of occlusion is sufficient to cause maximal observable damage to the brain tissue, and extending the occlusion time does not exacerbate the injury further. This finding is consistent with some results in existing studies, such as the one by Albadawi *et al*., which found that the initial ischemic period had the most significant effect on tissue damage in thoracic aorta I/R injury on spinal cord inflammation.[5]

Previous studies have shown that occlusion of the descending thoracic aorta leads to increased cardiac output and subsequent myocardial stress.[27,28] In our study, we observed similar pathological changes, including disorganized myocardial fibers and increased collagen deposition, particularly in the 1-h occlusion group. Our finding aligns with earlier research on the effects of TAO on cardiac tissue, reinforcing the notion that prolonged ischemia exacerbates myocardial injury.[29] Report showed that cross-clamping of the thoracic aorta was associated with marked decreases in blood flow and oxygen consumption in organs and tissues below the aortic occlusion. Above the aortic occlusion, blood flow increased but oxygen uptake decreased, which contributes to cardiac injury.[30] In addition, the systemic inflammatory response triggered by arterial occlusion also contributes to cardiac injury. The observed myocardial damage and collagen deposition are consistent with the inflammatory responses and fibrotic changes reported in other models of cardiac I/R injury.[31]

HyP inhibited both cardiac and cerebral damage and the systemic inflammatory response induced by thoracic aorta occlusion

These findings underscore the critical impact of TAO on various tissues, highlighting the need for effective therapeutic strategies to mitigate these injuries. One promising avenue of research is the use of HyP as a potential treatment to enhance tissue resilience and reduce the extent of damage caused by ischemic events.

Our study further explored the impact of HyP therapy on brain and heart tissue damage induced by TAO. The results demonstrate that HyP treatment significantly improved TAO-induced brain tissue damage, especially

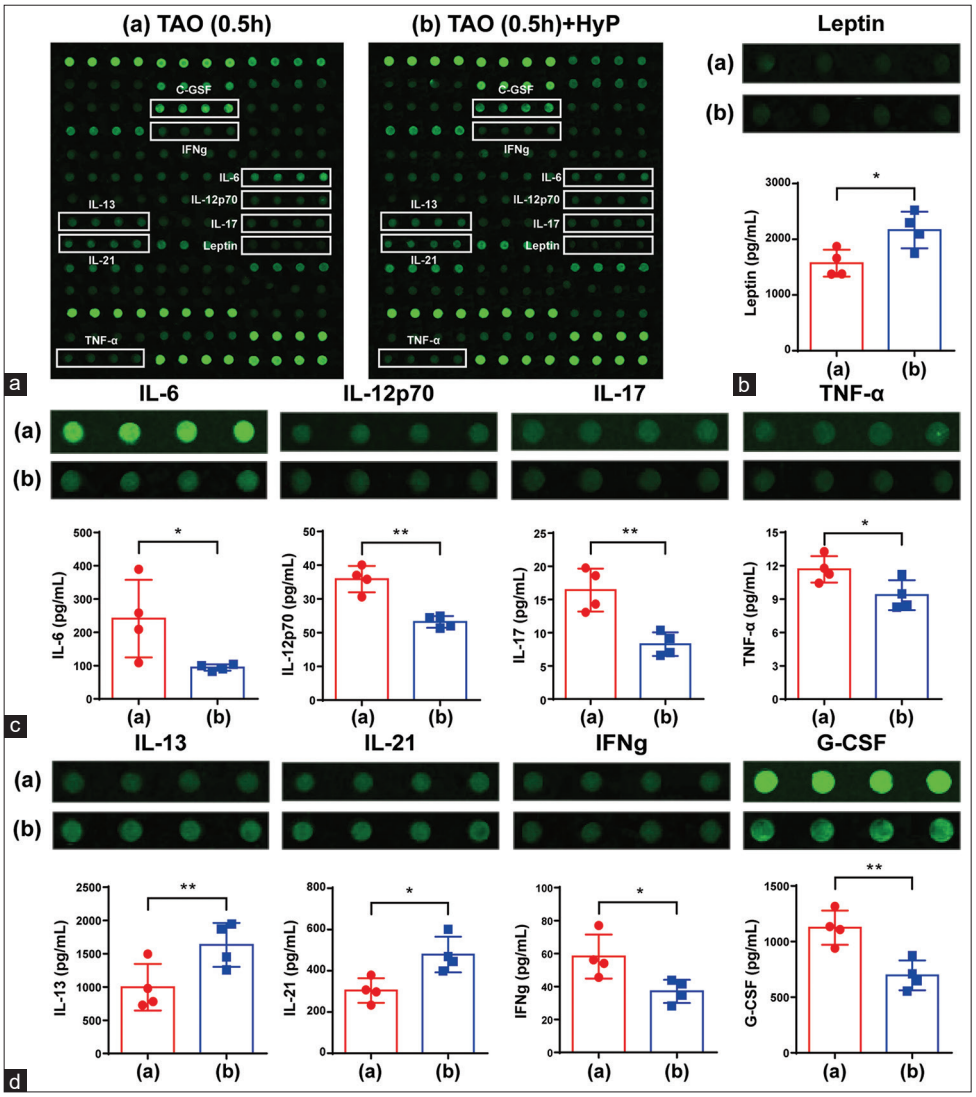

Figure 7: HyP therapy regulated the expression of inflammation-related proteins inflicted by thoracic aorta occlusion (TAO) (0.5 h) in the serum. (a) Representative images of the quantitative inflammation array of protein chip detection. (b) Representative images of expressed inflammation proteins of leptin. Data are presented as mean ± standard error of the mean (SEM), versus TAO group, **P* < 0.05, *n* = 4. (c) Representative images of expressed inflammation proteins: Interleukin-6 (IL-6), IL-12p70, IL-17, and tumor necrosis factor -α. Data are presented as mean ± SEM, versus TAO group, **P* < 0.05, ***P* < 0.01, *n* = 4. (d) Representative images of expressed inflammation proteins: IL-13, IL-21, interferon gamma, and Granulocyte colony-stimulating factor (G-CSF). Data are presented as mean ± SEM, versus sham group, **P* < 0.05, ***P* < 0.01, *****P* < 0.0001, *n* = 4

in the 0.5-h occlusion group where HyP treatment reversed the damage in the hippocampal CA1, CA3, and DG regions. We further observed that HyP treatment has varying efficacy depending on the duration of ischemic times. In the 0.5-h occlusion group, HyP treatment could reverse damage in the hippocampal CA1, CA3, and DG regions, possibly due to the lighter damage caused by the shorter ischemic time, allowing for stronger tissue repair abilities. In the 1-h occlusion group, significant improvement was observed only in the CA1 region. The longer ischemic time likely led to more severe damage and reduced tissue repair capabilities, limiting the overall effectiveness of HyP treatment. The unique sensitivity of the CA1 region to ischemic injury, due to its high metabolic demands and lower antioxidant capacity,[32] may explain why HyP treatment could still offer some protective effects in this region even during longer ischemic periods. Thus, the differential responsiveness of hippocampal subregions to HyP treatment underscores the complex interplay between ischemic duration and tissue repair mechanisms.

Furthermore, the protective effect of HyP on brain tissue is consistent with its protective effect on the heart, with variations depending on the duration of ischemia. Specifically, in the 0.5-h occlusion group, HyP treatment significantly reduced myocardial injury and collagen deposition. Although HyP treatment in the 1-h occlusion group also significantly reduced myocardial injury and collagen deposition, its effect was relatively weaker.

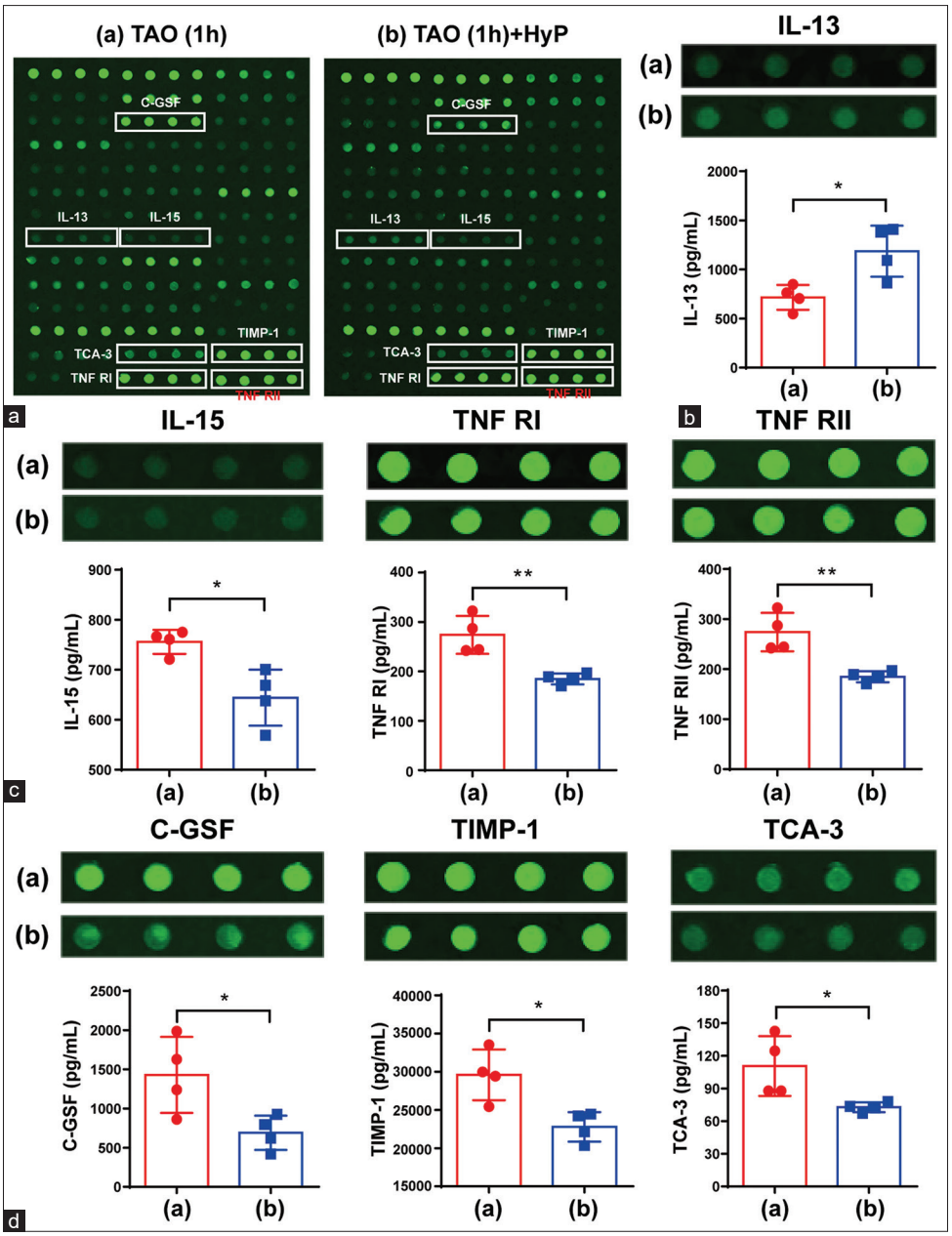

Figure 8: HyP therapy regulated the expression of inflammation-related proteins inflicted by thoracic aorta occlusion (TAO) (1 h) in the serum. (a) Representative images of the quantitative inflammation array of protein chip detection. (b) Representative images of expressed anti-inflammation proteins of IL-13. Data are presented as mean ± standard error of the mean (SEM), versus TAO group, **P* < 0.05, *n* = 4. (c) Representative images of expressed inflammation proteins: IL-15, tumor necrosis factor (TNF) RI, and TNF RII. Data are presented as mean ± SEM, versus TAO group, **P* < 0.05, ***P* < 0.01, *n* = 4. (d) Representative images of expressed inflammation proteins: Granulocyte colony-stimulating factor (G-CSF), Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) and T Lymphocyte-Secreted Protein I-309 (TCA-3). Data are presented as mean ± SEM, versus sham group, **P* < 0.05, *n* = 4

My study demonstrates that TAO induces an increase in inflammatory cytokines in the blood, and the regulatory effect of HyP treatment on these cytokines is noteworthy. IL-13, an anti-inflammatory cytokine, was found to increase, which may help mitigate the inflammatory response and tissue damage. Conversely, pro-inflammatory cytokines such as IL-6, IL-12p70, IL-17, and TNF-α were reduced, which could also contribute to alleviating inflammation and tissue injury. Previous studies have reported that plasma levels of TNF-α and IL-6 peak 1-4 h after thoracoabdominal aortic aneurysm repair,[33,34] and that IL-6 levels significantly increased with longer balloon occlusion times, particularly in the 60-min and 90-min occlusion groups, in the thoracic aortic REBOA Yorkshire swine model.[35] and that IL-6 levels significantly increased with longer balloon occlusion times, particularly in the 60-min and 90-min occlusion groups, in the thoracic aortic REBOA Yorkshire swine model.

The changes in the levels of these inflammatory cytokines in the blood can also serve as clinical biomarkers for diagnosing and monitoring TAO-induced injury. Specifically, the increase in the anti-inflammatory cytokine IL-13 and the decrease in pro-inflammatory cytokines IL-6, IL-12p70, IL-17, and TNF-α could be potential biomarkers for the efficacy of HyP treatment. These changes in serum inflammatory cytokines not only reflect the protective effects at the tissue level but also provide a convenient means to assess therapeutic efficacy and disease progression. Therefore, future research should further explore the feasibility and accuracy of these inflammatory cytokines as clinical biomarkers to better guide clinical treatment and decision-making.

In comparison to existing research, our study provides new evidence supporting the potential of HyP therapy in brain tissue protection. Li *et al*. (2017) reviewed the role of hypoxic preconditioning in neuroprotection, highlighting how it can protect tissues by regulating oxidative stress, inflammation, and cell death pathways.[16] Our findings align with this view, further suggesting that HyP treatment may mitigate I/R injury through similar mechanisms.[36]

The results of this study suggest that HyP therapy holds significant clinical potential in alleviating thoracic aortic I/R injury. By reducing tissue damage in key organs and modulating inflammatory responses, HyP therapy may improve patient outcomes. This finding provides important evidence for the development of new treatment strategies, particularly in clinical scenarios involving TAO, such as REBOA in cases of severe bleeding, aneurysm repair or aortic replacement surgeries, and coronary artery bypass grafting. Future studies should further investigate the mechanisms and optimal implementation protocols of HyP treatment to maximize its clinical value. In addition, these findings should be validated in larger-scale animal models and clinical trials to ensure the safety and efficacy of HyP treatment. Through these efforts, we aim to provide a novel and effective therapeutic option for patients with thoracic aortic I/R injury, thereby significantly improving their clinical outcomes.

In assessing the implications of our findings, however, several limitations of our study should be noted. While our murine model provides valuable insights, it may not fully encapsulate the breadth of human physiological and pathological complexities. The genetic uniformity of our mouse model contrasts with the genetic diversity observed in human populations, which could influence the response to HyP treatment. In addition, the controlled and acute nature of the TAO model in our study may not fully represent the spectrum of chronic and variable ischemic conditions encountered in clinical practice. These considerations suggest that our preclinical findings require further validation in more diverse and complex settings to establish the clinical effectiveness and safety of HyP treatment in humans. Despite these limitations, our study offers valuable preliminary evidence supporting the potential of HyP therapy in mitigating TAO-induced tissue damage and inflammation. Future studies, ideally conducted in larger animal models and eventually in human trials, will be crucial to extending our understanding and optimizing the application of HyP treatment for patients with thoracic aortic I/R injury.

Conclusion

Our study demonstrates that TAO leads to significant damage in remote organs, specifically the heart and brain. Hypoxic postconditioning (HyP) treatment significantly alleviated this damage, particularly with shorter ischemic durations, and reduced systemic inflammatory responses by modulating various cytokines. These findings highlight the potential clinical application of HyP treatment in mitigating TAO-induced tissue damage and inflammation. By reducing tissue damage and modulating inflammatory responses, HyP therapy may improve patient outcomes in clinical scenarios involving TAO. Future studies should further investigate the mechanisms and optimal protocols of HyP treatment to maximize its clinical value, ultimately providing a novel therapeutic option for patients with thoracic aortic I/R injury.

Author contributions

Methodology, J. X. and F. T.; Validation, J. X. and F. T.; Writing original draft, J. X. and C. H. R.; Writing Review and Editing, F. T. and C. H. R.; Investigation, J. X., S. J. L. Y. M. W. and W. B. Z.; Formal analysis, J. X. and F. T.; Conceptualization, F. Y. L., X. M. J. and C. H. R.; Supervision, X. M. J.; Project administration, C. H. R.

Animal experimentation approval

In this study, all experiments were conducted in accordance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Capital Medical University on October 14th, 2021 (approval number: 2021/255).

Data availability statement

The data used and analyzed in this study is available from the corresponding author upon reasonable request.

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Conflicts of interest

Xunming Ji is the Editor-in-Chief, Sijie Li and Wenbo Zhao are Associate Editors, Changhong Ren is an Editorial Board member of *Brain Circulation*. The article was subject to the journal's standard procedures, with peer review handled independently of them and their research groups.

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