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Efficacy of ozonated autohemotherapy for improvement of myocardial injury following traumatic brain injury

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Abstract

Background Traumatic brain injury is a kind of injury caused by external violence on the head. Its danger is not limited to life rescue in the early stage of the disease. Moreover, the subsequent inflammatory reaction and the change in its oxidative stress level will cause secondary myocardial injury. The purpose of this study is to explore the myocardial protective effect of ozone autohemotherapy (OA) in the progression of acute traumatic brain injury (TBI).

Methods Forty patients with acute TBI were recruited and divided into The treatment group (Group OA, $n=18$) and the Control group (Group C, $n=19$). Patients in Group OA received OA before surgery and on the 1st and 2nd postoperative days, while patients in Group C underwent autologous blood transfusion. Venous blood was collected from all patients before (T0) and after 7 days (T1) days of surgery for measurement of cardiac troponin T (cTnT) and amino-terminal pro-B-type natriuretic peptide (NT-proBNP). At T0 and T1, transthoracic cardiac ultrasound was performed to measure left ventricular ejection fraction (LVEF), tricuspid annular plane systolic excursion (TAPSE), and venous blood was sampled to determine the contents of superoxide dismutase (SOD) and malondialdehyde (MDA). NIH Stroke Scale (NIHSS) and Glasgow Coma Scale (GCS) scores were calculated, and other clinical indexes were recorded.

Results (1) The levels of cTnT at T1 were significantly higher as compared with that at T0 in both groups ($p<0.01$). Compared with Group C, a remarkable decline in the content of NT-proBNP was found in Group OA at T1 ($p=0.021$). (2) The LVEF ($p=0.002$) and serum SOD ($p=0.015$) at T1 were significantly increased in Group OA as compared with those in Group C. (3) The length of Intensive Care Unit and hospitalization time for patients in Group OA was distinctly shorter than that for patients in Group C ($p=0.021$, $p=0.015$, respectively).

Conclusion Perioperative OA treatment can alleviate the secondary myocardial injury during the disease course of TBI, which might be associated with its myocardial protective effect against oxidative stress.

Trial registration This study was approved by the Ethical Committee of Changzhou NO.2 People's Hospital. The protocol was registered prospectively with the Chinese Clinical Trial Registry (ChiCTR2000029612) on February 02, 2020.

Keywords Ozonated autohemotherapy, Acute traumatic brain injury, Myocardial protection, Oxidative stress

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Acute traumatic brain injury (TBI) is caused by external violence acting on the head, mainly presenting with a skull fracture, soft tissue injury, brain injury, etc. Since it has characteristics of critical illness, rapid development, and high disability, patients may benefit less from subsequent treatment if not treated early and effectively. If severe, they will be in a life-threatening condition. Except for the risk at the initial stage of the disease, there will be a series of biochemical and cellular changes during disease progression and pose risks, including inflammatory reaction, changes in self-oxidative stress level, and mitochondrial dysfunction [1–4]. In the later stage, patients can develop systemic inflammation and coagulation cascade, subsequently leading to sustained organ damage. Particularly, secondary myocardial injury following TBI is notably prevalent. According to the data from the Centers for Disease Control and Prevention (CDC), there were more than 220,000 hospitalizations and 64,000 deaths related to TBI in 2020 [5]. The incidence of secondary cardiovascular lesions following TBI is up to 77%, and it can potentially lead to myocardial ischemia, electrocardiogram (ECG) abnormalities, arrhythmia, acute myocardial infarction (AMI), etc.

Ozone (O3) is a molecule composed of three oxygen atoms (O) and has been clinically used for 100 years. Previous research showed that O3 could alleviate the pathological changes and functional impairment of the kidney induced by ischemia–reperfusion (I/R), and it could also significantly reduce the neurological score of patients with acute cerebral infarction. Other studies found that O3 therapy could protect the brain after acute TBI and facilitate the recovery of function and neurological behaviors of the brain during hospitalization [6, 7]. However, whether OA has protective effects against brain-derived myocardial injury remains fully understood. The present study aims to discuss the effect of OA on myocardial injury following acute TBI and explore the potential mechanism. The study results are expected to provide a new direction for developing protection measures against clinical myocardial injury following acute TBI.

Materials and methods

Patient samples

Forty-three patients who underwent surgery for acute TBI in Changzhou NO.2 People's Hospital between January 2020 and June 2021 were included in this study. Two groups were generated: The treatment group (Group OA, $n=18$) and the Control group (Group C, $n=19$). This study is a randomized controlled study. According to the literature and the pre-experimental results, the cTNT in group C was 0.02 ± 0.0088 after operation, which is expected to decrease by 0.01 in group OA.Set both sides$\alpha=0.05$, the degree of assurance is 90%, group C: group OA = 1:1.According to PASS15, 36 subjects are needed. Considering the 10% loss rate, 40 subjects need to be included(20 in each group). All patients were aged between 18 and 75 years old (gender unlimited) and diagnosed with acute TBI by medical history and brain computed tomography (CT) or magnetic resonance imaging (MRI). All patients were classified as grade III-IV according to the American Society of Anesthesiologists (ASA) grading system. All patients were confirmed with traumatic epidural hematoma, traumatic subarachnoid hematoma, and traumatic intra-cerebral hemorrhage (ICH). Patients were excluded from the study if they (1) previously had heart diseases, (2) had contraindications for OA, and (3) suffered from severe impairment of the liver and kidney. This study was approved by the Medical Ethical Committee of Changzhou NO.2 People's Hospital([2020]YLA053). All patients agreed to participate in this study and signed the Informed Consent.

OA treatment

Venous blood (150 ml) was sampled from the central venous catheter (CVC) on the day of surgery, the 1st and 2nd postoperative days. All blood samples were collected in disposable sterile vacuum bags containing anticoagulants to prevent blood clotting. O3 (25 μg/ml) was injected into the blood with a syringe at 1:1, followed by vibration in a gentle circular motion to obtain bright red oxygenated blood. Subsequently, the oxygenated blood was transferred back to the CVC at 100 drops/min with 25 min. The same experienced anesthesiologist blinded to the study completed the OA procedure. For Group C, blood samples (150 ml) were obtained at the corresponding time point and collected into disposable sterile vacuum blood bags that contain anticoagulants to prevent blood clotting. The mixture of blood and anticoagulants was infused into the patients' CVC.

Outcome measures

Blood indexes

Venous blood (5 ml) was sampled from all patients before (T0) and after 7 days (T1) of surgery. One blood sample was sent to the hospital's laboratory to measure the contents of cardiac troponin T and N-terminal pro-B-type natriuretic peptide, and the other blood sample was coagulated at room temperature for at least 30 min. After centrifugation at 4℃ and 3000 Revolutions per Minute for 10 min within 4 h, all samples were collected and immediately stored in the environment of -80℃. The contents of superoxide dismutase and malondialdehyde in the blood sample were tested according to the corresponding kit instructions.

Left ventricular ejection fraction (LVEF) and tricuspid annular plane systolic excursion (TAPSE)

LVEF was measured by transthoracic cardiac ultrasound (PHILIP, CX50). At T0 and T1, all patients underwent transthoracic cardiac ultrasound with an S5-1 probe, and the ejection fraction (EF) from four and two-chamber views was measured using the Simpson method, respectively. Measurements were repeated 3 times and averaged. TAPSE was measured according to the 2010 American Society of Echocardiography (ASE) guidelines [8]: in the standard apical four-chamber view, the sampling site was localized to the junction between the annulus of anterior leaflet of the tricuspid and the right ventricular free wall under M-ultrasound, and the maximum displacement of tricuspid annulus from the end-diastolic phase to the end-systolic phase was measured.

NIH Stroke Scale (NIHSS) score, Glasgow Coma Scale (GCS) score, and other clinical indexes

All patients' GCS and NIHSS scores were calculated at T0 and T1, respectively. The use of vasoactive drugs, rehydration, and urinary volume during surgery and Intensive Care Unit stay, the number of hypotensive episodes during surgery, pupil changes, recovery of consciousness, and complications during hospitalization were recorded.

Statistics

Statistical analysis was done with the IBM SPSS Statistics 23. Enumeration data conforming to normal distribution were expressed by mean ± standard deviation ($\bar{x} \pm s$) and compared using a t-test. Data with skewed distributions were shown as medians and interquartile ranges, and between-group and within-group comparisons were done using the Chi-square test or Whitney-Mann rank-sum test. Differences were considered statistically significant when $p < 0.05$.

Results

General data of patients

The flow diagram of recruitment through this study is shown in Fig. 1. A total of 40 patients were included, three of whom dropped out of the study(one case died, and two cases had second surgery). At last, 37 patients were included. The general data of patients included in this study are displayed in Table 1. In total, 37 acute TBI patients were enrolled, including 17 males aged 51—85 years old (mean: 71 years old) and 20 females aged 55—85 years old (mean: 67 years old). The degree of injury was mild-to-moderate in 11 patients (NIHSS < 16), severe in 12 patients (16 < = NIHSS < = 24), and very severe in 14 patients (NIHSS > 24). The CT results revealed cerebral parenchymal hemorrhage in 9 patients,

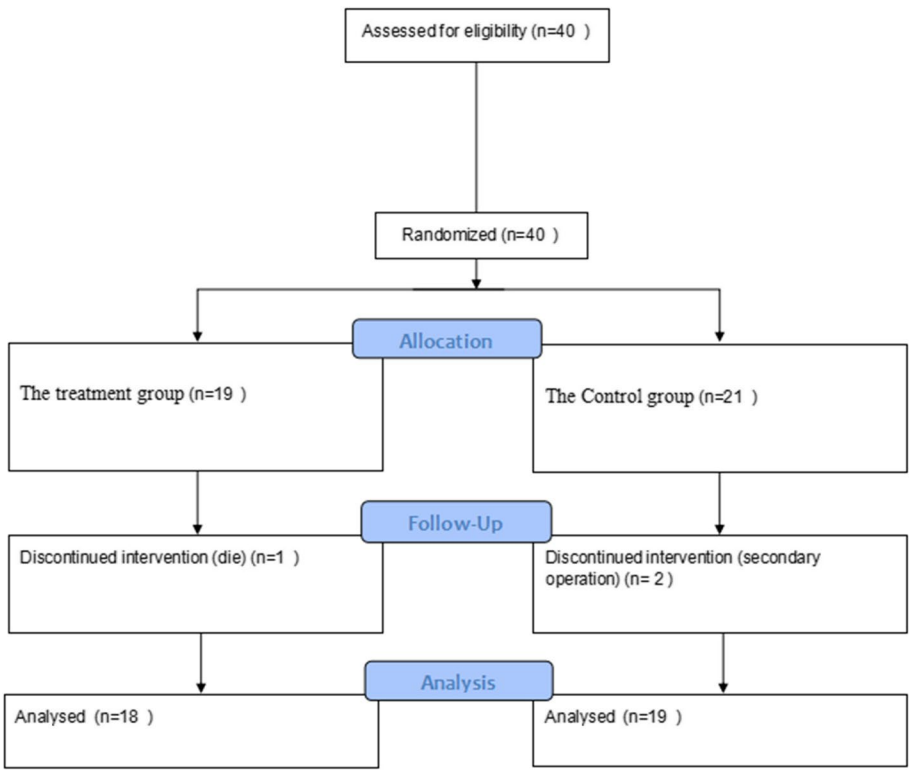

Fig. 1 CONSORT flow chart

Table 1 General data of patients. The data are expressed by the number of people and the mean standard deviation. The chi-square test was used for binary variables, and the test was used for continuous variables

	OA($n=18$)	C($n=19$)	p
Year	72.4 ± 10.1	69.1 ± 9.2	0.294
BMI	23.5 ± 3.0	23.7 ± 1.8	0.782
Sex			0.630
Male	9	8	
Female	9	11	
Potential disease			
Hypertension	12	13	0.909
Diabetes	4	1	0.132
Smoke	7	5	0.414
Alcohol	8	6	0.420
Clinical index			
GCS	9.0 ± 1.8	9.8 ± 2.0	0.520
NIHSS	22.2 ± 7.3	20 ± 8.5	0.313
WBC ($\times 10^3$/mL)	11.98 ± 1.88	11.83 ± 1.93	0.810
PLT ($\times 10^3$/mL)	301.5 ± 61.9	277.3 ± 45.7	0.182
Hb (gm/dl)	10.98 ± 1.09	10.9 ± 1.19	0.837
PT	11.18 ± 0.6	11.12 ± 0.63	0.739
APTT	25.29 ± 0.99	25.55 ± 1.17	0.483
ASA			0.248
III	7	11	
IV	11	8	
Intraoperative situation			
Fluid replacement	2055 ± 539	2052 ± 468	0.986
Urine volume	428 ± 159	418 ± 130	0.846
Hypotension frequency	2.6 ± 1.0	2.6 ± 0.9	0.920

epidural hemorrhage in 15 patients, and subarachnoid hemorrhage in 13 patients. All patients underwent surgery on the day of admission.

The GCS and NIHSS scores on admission showed no significant differences between Group C and Group OA. In addition, no significant differences were found between the two groups in terms of the biochemical indexes, including the counts of white blood cells (WBC), platelet (PLT) and hemoglobin (Hb), prothrombin time (PT), and activated partial thromboplastin time (APTT). There were also no differences between the two groups regarding the intraoperative rehydration volume, urinary volume, and number of hypotensive episodes. Intraoperative hypotensive episode is defined by invasive arterial blood pressure < 90/60 mmHg, which can be corrected within 3 min.

LVEF and TAPSE

The difference in the LVEF at T0 between the two groups was insignificant. At T1, the LVEF in both groups decreased significantly ($p<0.01$), and that in Group OA was significantly higher than in Group C ($p=0.002$, Fig. 2). Similarly, the TAPSE at T0 showed no distinct difference between the two groups. At T1, TAPSE reduction was found in both groups ($p<0.01$), and the level in Group OA was higher than that in Group C with no statistical significance ($p=0.126$, Fig. 3).

Serum levels of cTnT and NT-proBNP

There were no significant differences between the two groups regarding the serum levels of cTnT and NT-proBNP at T0. The serum cTNT level in the OA group was lower than in the C group at T1, and the difference was not statistically significant ($p=0.07$). The serum NT-proBNP level in the OA group was significantly lower than in the C group at T1, and the difference was statistically significant ($p=0.021$, Figs. 4 and 5).

Enzyme activities of SOD and levels of MDA

No distinct differences regarding the serum enzyme activities of SOD and levels of MDA at T0 between the two groups were found. At T1, the serum enzyme activities of SOD increased, while the levels of MDA decreased in both groups ($p<0.01$). In comparison to Group C, Group OA had higher enzyme activities of SOD ($p=0.015$) but lower levels of MDA ($p=0.016$) at T1 (Figs. 6 and 7).

Clinical indexes

NIHSS and GCS scores of all patients were calculated at T1, and no statistically significant differences were found between the two groups. The time to recovery of consciousness in Group OA was shorter than that in Group C, and the difference was not significant. Additionally, the length of the Intensive Care Unit ($p=0.021$) and hospital ($p=0.015$) stay in Group OA was shorter than in Group C.

During hospitalization, complications occurred in 1 patient in Group OA, including one with pulmonary and urinary tract infections. In Group C, 6 patients had complications, including impairment of consciousness in 3 patients, pulmonary infection in 2 patients, and urinary tract infection in 1 patient. See Table 2 for more details.

Discussion

Acute TBI disrupts the integrity and function of the brain and causes systemic multi-system injury and dysfunction via neuroendocrine and neuroimmune mechanisms, including dysfunction of the cardiovascular, respiratory, immune, endocrine, and hematology systems. There is an increasing body of evidence that substantiates the physiological and pathophysiological interplay between the nervous and cardiovascular systems. More than 1.5

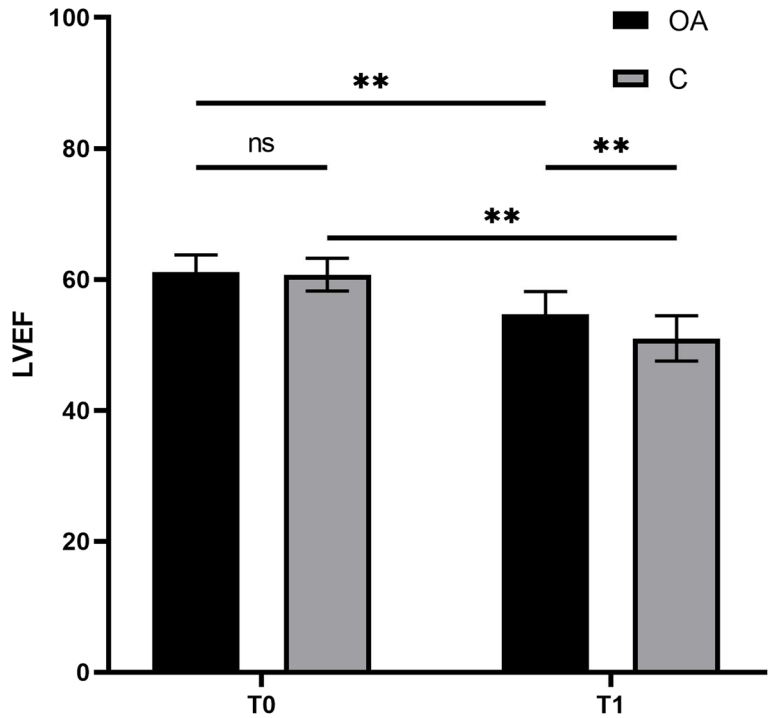

Fig. 2 Comparison of ejection fraction between the two groups. Data is represented as mean ± SD, $n = 18$ OA group, $n = 19$ C group. *$p < 0.05$, **$p < 0.01$, ns, not significant

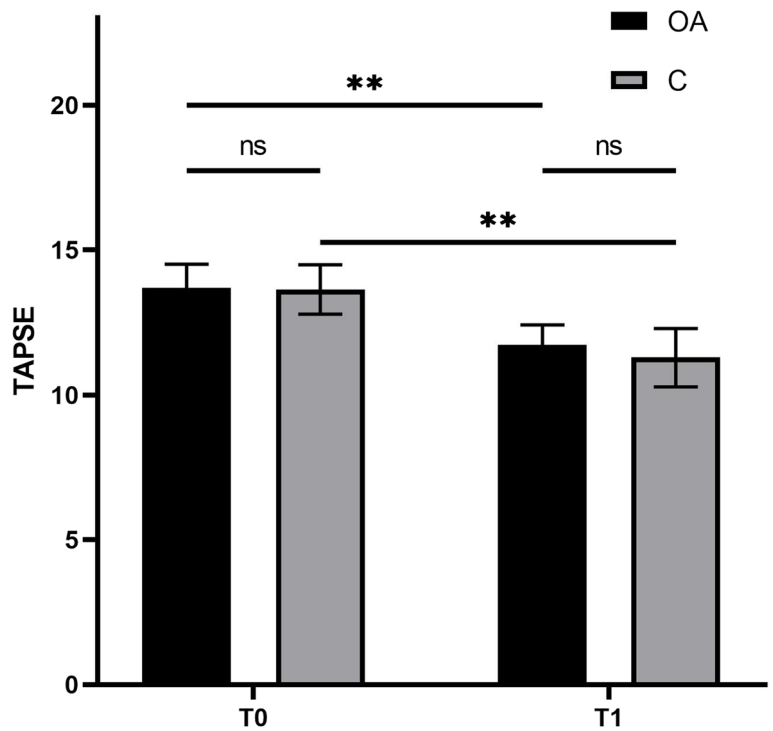

Fig. 3 Comparison of tricuspid annulus displacement between the two groups. Data is represented as mean ± SD, $n = 18$ OA group, $n = 19$ C group. *$p < 0.05$, **$p < 0.01$, ns, not significant

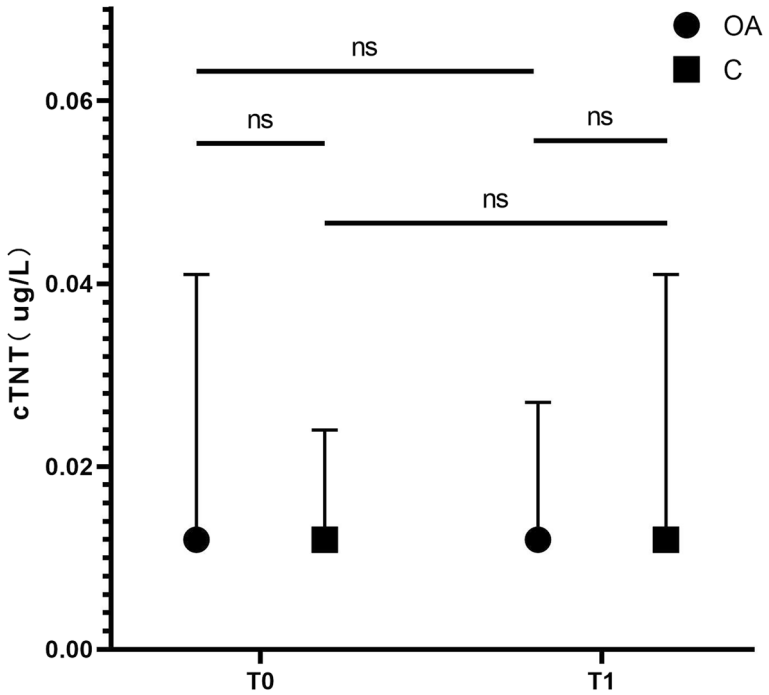

Fig. 4 Comparison of serum cTNT levels between the two groups. Data is represented as median [quartile], $n = 18$ OA group, $n = 19$ C group. $^{*}p < 0.05$, $^{**}p < 0.01$, ns, not significant

million deaths worldwide are related to neurocardiogenic mechanisms, including post-stroke cardiovascular complications, sudden death in epilepsy, Takotsubo syndrome (TTS), and neurogenic sudden cardiac death [9–13]. While not all TBI patients meet the diagnostic criteria for TTS, their myocardium also suffers varying degrees of impairment. Throughout the progression of the disease, patients may exhibit various manifestations, including, but not limited to, electrocardiographic alterations, anomalies in myocardial-related biomarkers, and abnormalities in cardiac motion. The above changes will exacerbate neurological impairment and increase mortality in patients with acute brain injury.

LVEF refers to the percent ventricular end-diastolic volume taken by stroke volume, and it is a characteristic of ventricular ejection measured from the volume view, a common clinical index of the left ventricular function. Thus, it can effectively evaluate the left ventricular systolic function. In the present study, compared with the control group, the level of LVEF in the postoperative treatment group was significantly higher. This result proves that OA treatment could significantly improve the postoperative contractile function of the heart in patients with acute TBI, especially the systolic function of the left ventricle, which may be related to ozone therapy improving myocardial cell function and strengthening myocardial contractility. The study of Carlsson et al. [14] reported that the right ventricular longitudinal strain contributed to approximately 75% of the right ventricular systolic function. The longitudinal displacement of the right ventricle, i.e., the systolic displacement of the tricuspid annulus toward the apex, could be used as an index to assess the right ventricular systolic function. However, there was no significant difference in TAPSE level between the two groups after the operation, which may be related to the fact that the right heart is mainly responsible for systemic blood recovery and volume.

Cardiac troponins (cTn) are regulators of myocardial contraction that are mainly composed of three subunits: cTnT (the most widely used in clinic), cardiac troponin I (cTnI), and cardiac troponin C (cTnC). Research revealed that the serum cTnT concentration is low under normal circumstances, and it increases following cardiomyocyte necrosis as a small portion of cTnT free in cardiomyocytes enter the blood through the cell membrane. Thus, it was believed that the cTnT concentration can reflect the degree of myocardial defect to some extent [15]. NT-proBNP is a polypeptide mainly arising from the ventricles and having multiple physiological functions, such as pressure lowering and vasodilation. It is released in response to pressure and volume overload, and thus, it can be used to reflect myocardial function and the extent of injury. In addition, it is related to the severity of heart failure and, therefore, instructive for the treatment of

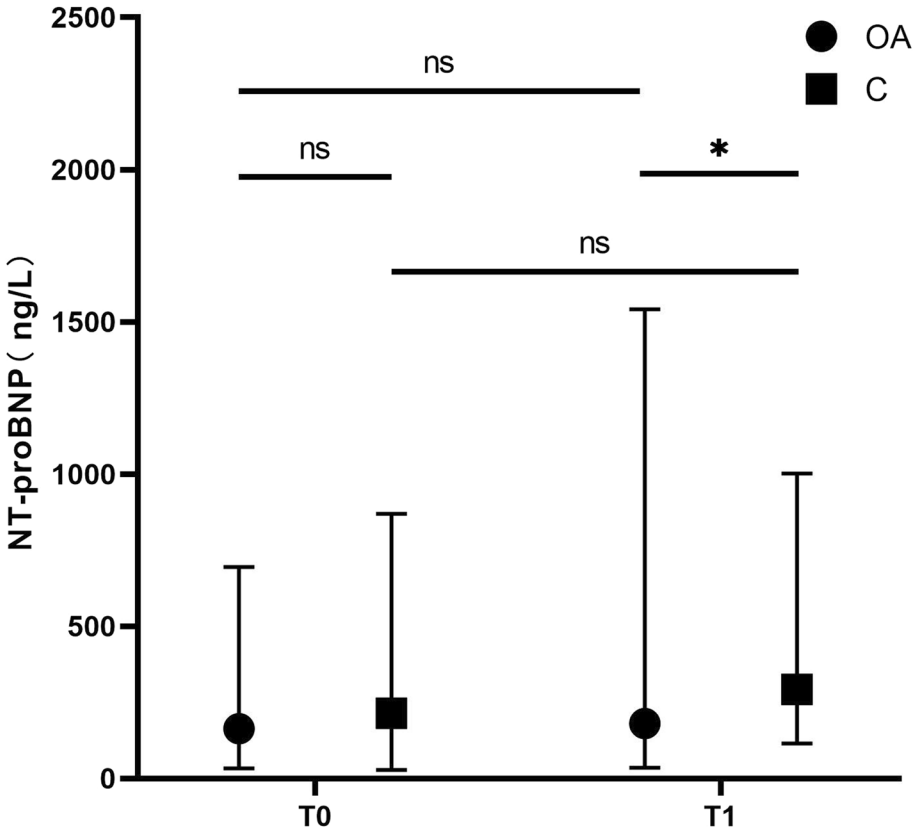

Fig. 5 Comparison of serum NT-proBNP levels between the two groups. Data is represented as median [quartile], $n = 18$ OA group, $n = 19$ C group. *$p < 0.05$, **$p < 0.01$, ns, not significant

heart failure and improvement of prognosis [16]. Our study found that OA treatment alleviated myocardial injury in patients with acute TBI, and reductions were found in the serum levels of cTnT and NT-proBNP after 7 days of surgery.

Under normal physiological conditions, there is a steady-state balance between the formation of oxygen free radicals and their removal by endogenous scavengers [17], while oxidative stress reflects the imbalance between peroxidation and antioxidation. On the one hand, the production and accumulation of reactive oxygen species(ROS) lead to systemic changes in the body; on the other hand, the body detoxifies itself and repairs oxidative stress-induced injuries. Recent studies reported that oxidative stress is ubiquitous during myocardial injury, and it is initially caused by the compromised cardiac oxygen and energy supply induced by absolute and relative hypoxia–ischemia (HI). It has been considered that the oxidative stress induced by excessive ROS production plays a crucial role during myocardial injury following acute TBI [6, 18, 19]. Related studies have confirmed that ROS and mitochondrial homeostasis changes are relevant in metabolic, inflammatory, and neurodegenerative diseases [20–23], and high-intensity exercise can lead to cardiac inflammatory response, increased infarct size, and decreased cardiac function. MDA is a product of lipid oxidation that can reflect the extent of lipid peroxidation. Lactate dehydrogenase (LDH) is released to the cytosol only when cells are damaged. In contrast, an increased enzyme activity of SOD, a protective factor with an antioxidant effect, indicates a stronger ROS scavenging capability of the body [24, 25]. As the final product of lipid oxidation, MDA will cause cross-linking polymerization of life macromolecules such as protein and nucleic acid, which is cytotoxic. The changes in SOD activity and MDA content can be used to evaluate the extent of oxidative stress-induced myocardial injury and drug efficacy. In the early stage of trauma, due to systemic inflammatory reaction, the ROS content in the body will increase sharply, leading to excessive oxidative stress, which will cause myocardial cell damage. The enzyme SOD scavenges superoxide anion radicals in vivo and can scavenge ROS, including superoxide anion radicals. However, when ROS is enzymolyzed, excessive SOD will make lipid peroxidation and destroy the cell membrane. The results of the present

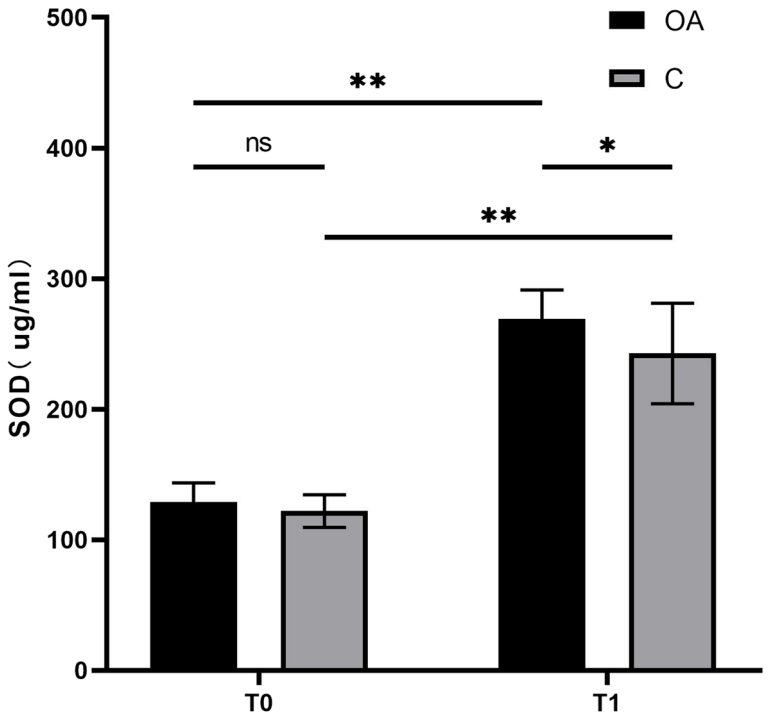

Fig. 6 Comparison of serum SOD content between the two groups. Data is represented as mean ± SD, $n = 18$ OA group, $n = 19$ C group. *$p < 0.05$, **$p < 0.01$, ns, not significant

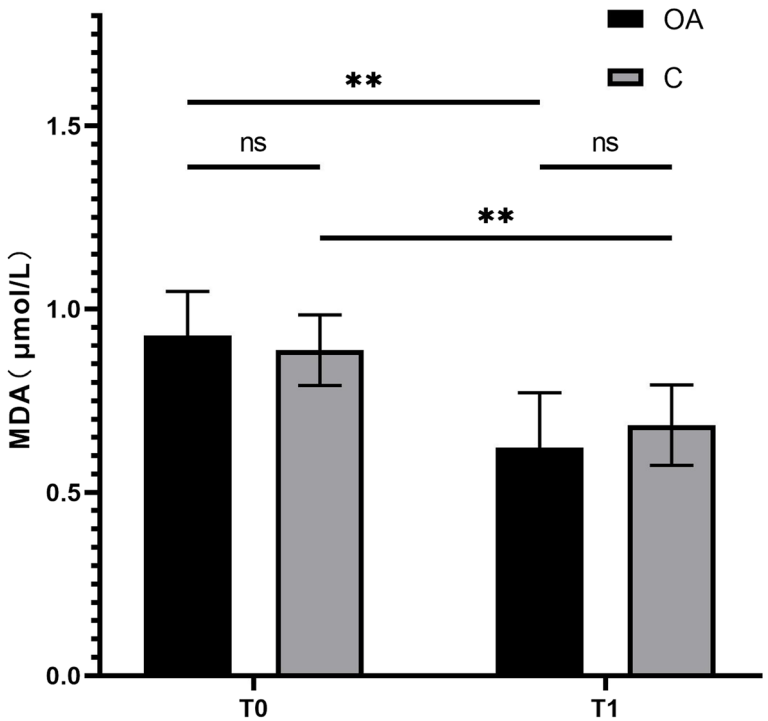

Fig. 7 Comparison of serum MDA content between the two groups. Data is represented as mean ± SD, $n = 18$ OA group, $n = 19$ C group. *$p < 0.05$, **$p < 0.01$, ns, not significant

Table 2 Comparison of clinical-related indexes between two groups of patients

	n	NIHSS	GCS	Time of consciousness recovery	Complication	ICU time	LOS
OA	18	24[15, 29]	8[7.8,11]	2[1,3.5]	1	16.3 ± 7.1	3.9 ± 4.4
C	19	23[17.27]	10[9, 11]	3[1, 5]	6	22.4 ± 8.3	8.6 ± 6.5
p		0.038	0.051	0.113	0.090	0.021	0.015

Complications were described by the number of cases, and other data were described by mean standard deviation and median [quartile]

study showed that OA treatment increased the serum enzyme activities of SOD and accelerated the removal of excessive ROS in the early stage. At the same time, OA treatment can reduce MDA content and protect cell membranes, although the difference is not statistically significant compared with the control group. Thus, we can infer that the OA treatment may reduce free radical damage by regulating the enzyme activities of SOD and MDA, thereby exhibiting myocardial protective effects.

Ozone reverses oxidative stress-induced damage, eliciting the upregulation of antioxidant enzymes and restoring redox balance in cells and organs, probably through a similar mechanism used for ischemic heart preconditioning [26–28]. Other studies have shown that pretreatment with multiple applications of low-dose ozone might increase the mRNA and protein expression levels of Nuclear factor erythroid-2 related factor 2 (Nrf2) in the hearts of rats and the mRNA expression levels of antioxidant enzymes (SOD1, SOD2) [29], which is consistent with our results. In addition, Ding et al. found that ozone pretreatment alleviated ischemia/reperfusion injury-induced myocardial ferroptosis by activating the Nrf2/Slc7a11/Gpx4 axis [30]. Nrf2 is a transcription factor that controls cellular defense responses to toxic and oxidative stress by modulating the expression of genes involved in antioxidant response and drug detoxification [31]. Whether Nrf2 plays a key role in OA's myocardial protection in patients with acute TBI needs further study.

This study also presents several limitations. First, the disease severity of participants in this study was not screened, resulting in a big difference in disease severity between patients, i.e., a big difference in NIHSS score and, in turn, a big difference in the subsequent disease progression. Secondly, the more intense trauma will be the impact of the inflammatory insult mediated by the oxidative species. Nevertheless, Our study lacks systemic inflammatory markers to reflect the severity of patients' systemic injury, and we hope to improve this in subsequent studies. Finally, the cTnT data we obtained were from the Department of Clinical Laboratory and recorded as 0.012 when the actual value was smaller than 0.012, which resulted in data bias.

To sum up, OA treatment can significantly improve the postoperative cardiac function of patients with acute TBI by increasing LVEF and decreasing the relevant markers of myocardial injury. Specifically, OA treatment enhances the body's anti-oxidative stress capability by increasing the serum enzyme activities of SOD, thereby facilitating the scavenging of ROS during disease progression. In clinical practice, OA treatment can accelerate the recovery after surgery, decrease the incidence of complications, reduce the length of ICU stay, and eventually accelerate rehabilitation. This treatment strategy can also provide a new direction for myocardial protection in patients with acute TBI.

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Authors' contributions
WCH is responsible for designing the experimental process, collecting and processing data, and writing and delivering papers. ZY is responsible for sorting out data, revising papers and consulting literature. LW and RLY are responsible for revising papers and guiding the delivery of papers. WZQ is responsible for guiding the design experiment, sorting out the data and modifying the paper. CJL is responsible for guiding and sorting out data and delivering papers.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethical approval for this study was provided by the Ethical Committee of Changzhou No. 2 People's Hospital (Number: [2020]YLA053). Written informed consent was obtained from all patients before participation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Lenzlinger PM, Morganti-Kossmann MC, Laurer HL, McIntosh TK. The duality of the inflammatory response to traumatic brain injury. Mol Neurobiol. 2001;24:169–81.
2. Zhang X, Chen Y, Jenkins LW, et al. Bench-to-bedside review: Apoptosis/programmed cell death triggered by traumatic brain injury. Crit Care. 2005;9:0.
3. Chong ZZ, Li F, Maiese K. Oxidative stress in the brain: novel cellular targets that govern survival during neurodegenerative disease. Prog Neurobiol. 2005;75:0.
4. Velmurugan GV, Hubbard WB, Prajapati P, et al. LRP1 deficiency promotes mitostasis in response to oxidative stress: implications for mitochondrial targeting after traumatic brain injury. Cells. 2023;12:1445.
5. Centers for Disease Control and Prevention. National Center for Health Statistics: Mortality data on CDC WONDER. 2023. Available online: https://wonder.cdc.gov/mcd.html. Accessed on 01 Apr 2023.
6. Gaur V, Aggarwal A, Kumar A. Protective effect of naringin against ischemic reperfusion cerebral injury: possible neurobehavioral, biochemical and cellular alterations in rat brain. Eur J Pharmacol. 2009;616:147–54.
7. Hemfindez F, Mendndez S, Gtmez M, Eng L. Efecto de la ozonoterapia intravascular sobre el sistema de la glutation peroxidasa. Rev CENIC Ciencias Biol. 1989;20:37–40.
8. Rudski LG, Lai WW, Afilalo J, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography Endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. J Am Soc Echocardiogr. 2010;23(7):685–713. https://doi.org/10.1016/j.echo.2010.05.010.
9. Feigin VL, Roth GA, Naghavi M, et al. Global burden of stroke and risk factors in 188 countries, during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Neurol. 2016;15:913–24.
10. Sposato LA, Lam M, Allen B, Richard L, Shariff SZ, Saposnik G. First-ever ischemic stroke and increased risk of incident heart disease in older adults. Neurology. 2020;94:e1559–70.
11. Thom M, Boldrini M, Bundock E, Sheppard MN, Devinsky O. Review: the past, present and future challenges in epilepsy-related and sudden deaths and biobanking. Neuropathol Appl Neurobiol. 2018;44:32–55.
12. Pelliccia F, Pasceri V, Patti G, et al. Long-term prognosis and outcome predictors in Takotsubo syndrome: a systematic review and meta-regression study. J Am Coll Cardiol HF. 2019;7:143–54.
13. Sposato Luciano A, Hilz Max J, Aspberg S, et al. Post-stroke cardiovascular complications and neurogenic cardiac injury: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;76:2768–85.
14. Carlsson M, Ugander M, Heiberg E, et al. The quantitative relationship between longitudinal and radial function in left, right, and total heart pumping in humans. Am J Physiol Heart Circ Physiol. 2007;293(1):H636–44.
15. Lee SM, Hutchinson M, Saint DA. The role of Toll-like receptor 4(TLR4)in cardiac ischaemic-reperfusion injury, cardioprotection and preconditioning. Clin Exp Pharmacol Physiol. 2016;43(9):864–71.
16. Misra A, Deswal A. NT-proBNP-guided and conventional therapies did not differ for readmission or mortality in acute decompensated HF. Ann Intern Med. 2018;169(4):JC21. https://doi.org/10.7326/ACPJC-2018-169-4-021. PMID: 30128511.
17. Choi MS, Do KM, Park YS, et al. Effect of naringin supplementation on cholesterol metabolism and antioxidant status in rats fed high cholesterol with different levels of vitamin E. Ann Nutr Metab. 2001;45:193–201.
18. Xia Y, Zweier JL. Substrate control of free radical generation from xanthine oxidase in the postischemic heart. J Biol Chem. 1995;270:18797–803.
19. O'Neil W, Timmis G, Bourdillon P, Lai P, Ganghadarhan V, Walton J, Ramos R, Lanfer N, Gordon S, Schork MA, Pitt B. A prospective randomized clinical trial of intra-coronary streptokinase versus coronary angioplastic therapy of acute myocardial infarction. N Engl J Med. 1986;314:812–28.
20. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders—a step towards mitochondria based therapeutic strategies. Biochim Biophys Acta. 2017;1863:1066.
21. Marchi S, Guilbaud E, Tait SWG, Yamazaki T, Galluzzi L. Mitochondrial control of inflammation. Nat Rev Immunol. 2022;23:159–73.
22. Johri A, Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. J Pharmacol Exp Ther. 2012;342:619.
23. Hiebert JB, Shen Q, Thimmesch AR, Pierce JD. Traumatic brain injury and mitochondrial dysfunction. Am J Med Sci. 2015;350:132–8.
24. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev. 2009;29(4):646–82.
25. Valacchi G, Bocci V. Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. Mediators Inflamm. 1999;8(4–5):205–9.
26. Barber E, Menendez S, Leon OS, Barber MO, Merino N, Calunga JL, Cruz E, Bocci V. Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia. Mediators Inflamm. 1999;8:37–41.
27. Leon OS, Menendez S, Merino N, Castillo R, Sam S, Perez L, Cruz E, Bocci V. Ozone oxidative preconditioning: a protection against cellular damage by free radicals. Mediators Inflamm. 1998;7:289–94.
28. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986;74:1124–36.
29. Meng W, Xu Y, Li D, Zhu E, Deng L, Liu Z, Zhang G, Liu H. Ozone protects rat heart against ischemia-reperfusion injury: a role for oxidative preconditioning in attenuating mitochondrial injury. Biomed Pharmacother. 2017;88:1090–7.
30. Ding S, Duanmu X, Xu L, et al. Ozone pretreatment alleviates ischemiareperfusion injury-induced myocardial ferroptosis by activating the Nrf2/Slc7a11/Gpx4 axis. Biomed Pharmacother. 2023;165:11939.
31. Mata A, Cadenas S. The antioxidant transcription factor Nrf2 in cardiac ischemia-reperfusion injury. Int J Mol Sci. 2021;22:11939.

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