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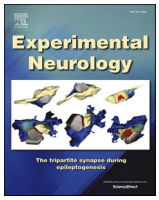

Research paper

DDAH1 deficiency exacerbates cerebral vascular endothelial dysfunction by aggravating BBB disruption and oxidative stress in thoracic blast-induced brain injury

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ABSTRACT

As terrorist incidents and underground explosion events have become more frequent around the world, brain injury caused by thoracic blast exposure has been more highlighted due to its injured organ, subsequent social and economic burden. It has been reported dimethylarginine dimethylaminohydrolase 1 (DDAH1) plays important roles in regulating vascular endothelial injury repair and angiogenesis, but its role in thoracic blast-induced brain injury remains to be explained. This study seeks to investigate the mechanism of DDAH1 on thoracic blast-induced brain injury. 40 C57BL/6 wild type mice and 40 DDAH1 knockout mice were randomly and equally divided into control group and blast group, respectively. The integrity of blood-brain barrier (BBB) was detected by Evans blue test. The serum inflammatory factors, nitric oxide (NO) contents, and asymmetric dimethylarginine (ADMA) levels were determined through ELISA. HE staining and reactive oxygen species (ROS) detection were performed for histopathological changes. Western blot was used to detect the proteins related to oxidative stress, tight junction, focal adhesion, vascular endothelial injury, and the DDAH1/ADMA/eNOS signaling pathway. DDAH1 deficiency aggravated thoracic blast-induced BBB leakage, inflammatory response, and the increased levels of inflammatory-related factors. Additionally, DDAH1 deficiency also increased ROS generation, MDA and IRE-α expression. Regarding cerebral vascular endothelial dysfunction, DDAH1 deficiency increased the expression of MCAM, FN1, LIMK1, VEGF, MMP9, Vimentin and N-cadherin, while lowering the expression of FMR1, Occludin, claudin-3, claudin-5, Lyn, LIMA1, Glrb, Sez6, Dystrophin, and phosphorylation of VASP. Also, DDAH1 deficiency exacerbated explosion-induced increase of ADMA and decrease of eNOS activity and NO contents. Thus, we conclude that DDAH1 could prevent cerebral vascular endothelial dysfunction and related injury by inhibiting ADMA signaling and increasing eNOS activity in thoracic blast induced brain injury.

1. Introduction

The global surge in terrorist attacks and underground explosions has resulted in considerable fatalities and economic damage, positioning blast injuries as a critical public health issue (Wolf et al., 2009; Liu et al., 2020). Blast injury, a kind of complex physical injury, is caused by the energy of overpressure waves hitting on the body. It is characterized by external slight and internal serious, rapid and complex traumatic progression, multiple important organs involved, leading to secondary serious injuries or immediate death (Pearce et al., 2017). In injured organs affected by blast overpressure, the lung has been viewed as the prime site and the research emphasis (Li et al., 2020). However, most studies concentrate on the direct impact of blast waves on the lungs, frequently neglecting injury to other organs. As the research evolves, brain injury caused by thoracic blast exposure has been more highlighted due to its injured organ, subsequent long-term social and economic burden (Phipps et al., 2020). Besides mechanical transmission through the vasculature, brain injury caused by thoracic blast is closely linked to the systemic inflammatory response triggered by blast lung injury (BLI) (Cong et al., 2021). Despite these findings, the precise mechanisms of thoracic blast-induced brain injury remain inadequately understood and require further investigation.

ADMA, an endogenous nitric oxide synthase (NOS) inhibitor, impairs endothelial NOS (eNOS) activity, thereby reducing NO bioavailability and increasing ROS generation (Chen et al., 2020). Elevated ADMA levels have been implicated in several pathological conditions, including

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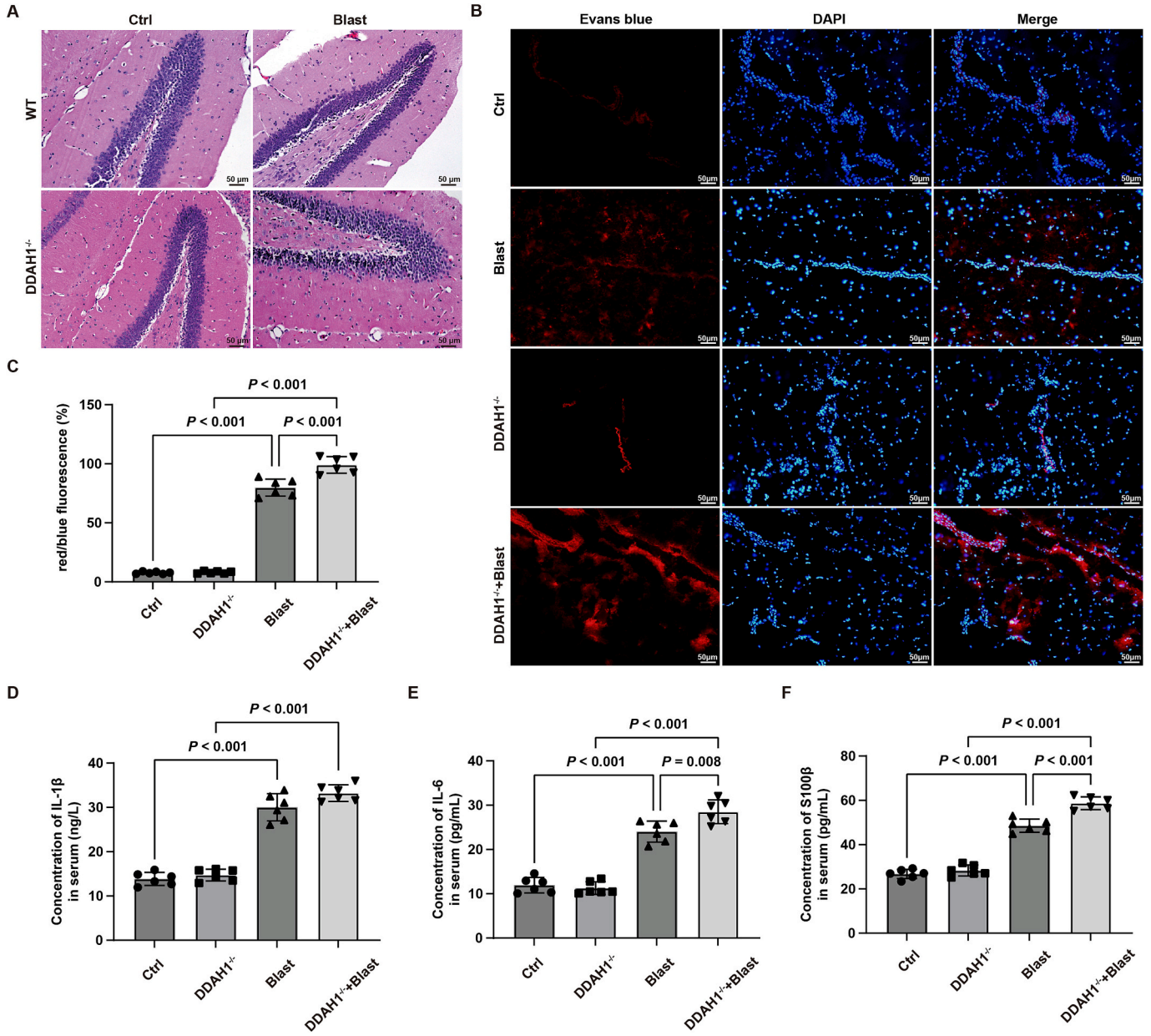

Fig. 1. DDAH1 knockout exacerbated inflammation and blood-brain barrier damage in thoracic blast-induced brain injury.
(A) HE stains of brain tissue. (B) Evans blue test under fluorescence microscope. (C) Evans blue fluorescence area statistics. (D—F) Levels of IL-1β(D), IL-6(E), and S100β(F) in serum. Data are mean ± SD. $n = 6$ for each group in each experiment.

hypertension, diabetes, cardiac dysfunction and vascular injury. DDAH1, the primary isoenzyme in the decomposition of ADMA, plays a crucial role in regulating endothelial injury repair and angiogenesis by maintaining NO signaling and degrading ADMA. DDAH1 deficiency significantly increases ADMA levels, accelerates cell oxidation and apoptosis (Almazroue et al., 2023). Reddy et al. demonstrated that DDAH1 overexpression enhances endothelial cell proliferation by reducing ADMA levels, increasing NO production, and upregulating key angiogenic markers such as CD31, VEGF, and HIF-1 (Reddy et al., 2018). In our previous study, we observed that DDAH1 knockout exacerbated leukocyte transendothelial migration, intensifies oxidative stress, and aggravates endothelial barrier dysfunction in murine models of BLI (Cong et al., 2022). However, the role of the DDAH1/ADMA/eNOS signaling pathway in brain injury caused by thoracic blast exposure has not been reported yet. To address this gap, we established thoracic blast induced brain injury models in C57BL/6 mice and DDAH1 knockout ($DDAH1^{-/-}$) mice. We assessed the expressions of proteins associated with BBB permeability, tight junction (TJ), and endothelial injury, aiming to elucidate the role of the DDAH1/ADMA/eNOS signaling pathway in thoracic blast-induced brain injury.

2. Materials and methods

2.1. Animal and experimental protocols

Forty $DDAH1^{-/-}$ mice were obtained from Jackson Laboratory (Sacramento, CA) and same amount of C57BL/6 wild-type (WT) mice were provided by the Beijing Vital River Laboratory Animal Technology Limited Company, P.R. China. Each kind of mice equally divided into two groups, control group(Ctrl) and blast group(Blast). All mice were kept in a room maintaining a temperature of 20 ± 2 °C and humidity of 55–65 %, and allowed free access to food and water in their cages. After

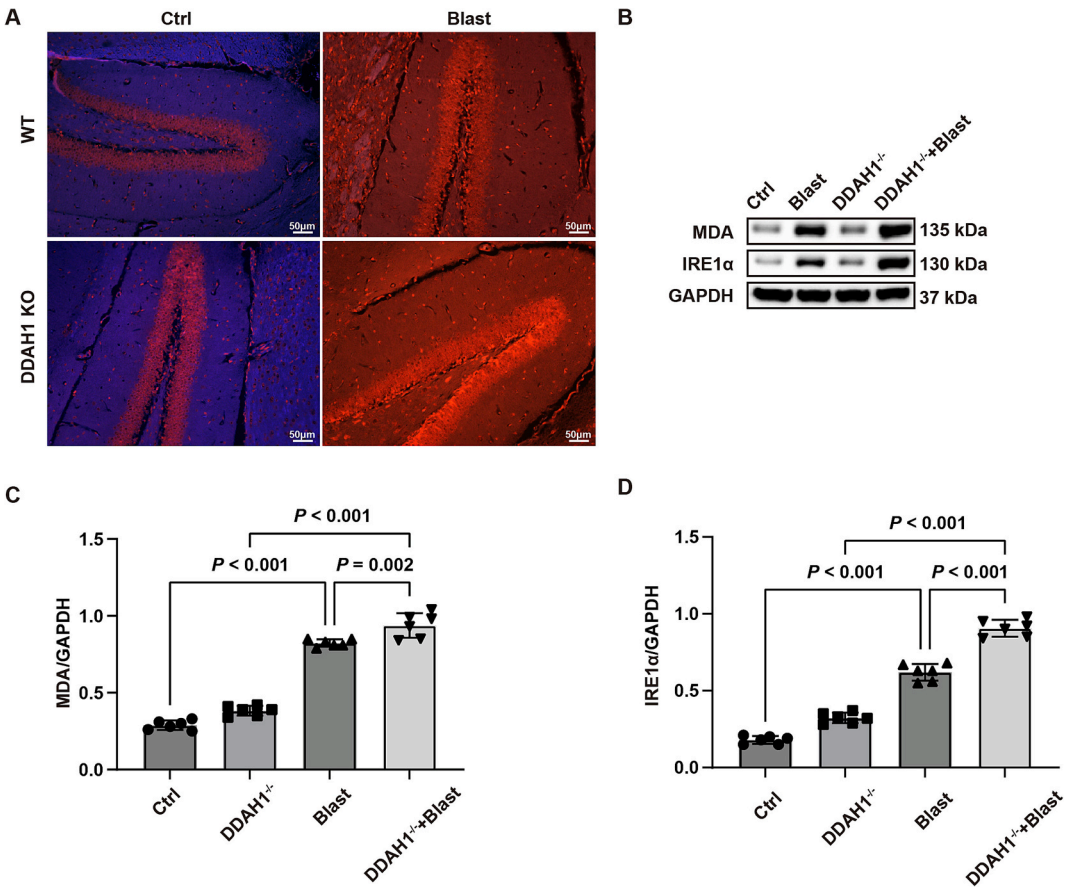

Fig. 2. ROS generation and oxidative stress-related protein changes in the brain tissue after thoracic blast exposure.
(A) ROS generation of brain tissue. (B) Western blot of IREα and MDA in each group. (C) Relative density of MDA. (D) Relative density of IREα. Data are mean ± SD. $n = 6$ for each group in each experiment.

acclimation, WT and DDAH1$^{-/-}$ mice in blast group will be exposed to explosion on chest, and samples will be collected at 48 h. Animal welfare and experimental design was approved by the Ethics Committee of the General Hospital of Northern Theater Command.

2.2. Thoracic blast model

Thoracic blast model was established as previously described (*Chitosan Oligosaccharide Ameliorates Acute Lung Injury Induced by Blast Injury through the DDAH1/ADMA Pathway - PubMed*, 2024). Briefly, mice were anesthetized by the abdominal injection of 2 % pentobarbital sodium (1.5 ml/kg). Aluminum foils were placed in the middle of main steel pipe and upper steel pipe and were fixed by screws. The anesthetized mice were placed on rubber pad with their chest exposed. By using an air pressure pump, the air pressure in the lower part of the device was increased until the burst of aluminum foil. The compressed air rapidly expanded from the blasting port at high speed, forming shock waves that impacted the chest of the mouse. The pressure detected by a pressure sensor is transmitted through a data cable connected to a computer, the pressure waveform was obtained by the formula: pressure (PSI) = voltage value*1000/50.08. The instantaneous shock wave overpressure was 321 ± 24 PSI in this experiment. The diagram of blast injury simulation device was shown in Supplymentary Fig. 1.

2.3. Sample collection and processing

After 12 h of fasting and 4 h of water deprivation preoperatively, mice were intraperitoneally anesthetized with 2 % pentobarbital sodium (1.5 ml/kg). Serum collection was for enzyme-linked immunosorbent assay (ELISA). Six mice of each group were for Evans blue stain. For the others, half of each brain was immersed in 10 % formalin buffer for histological analysis, and the remaining fresh brain tissue was placed in −80 °C refrigerator for protein determination.

2.4. Elisa

Levels of NO content and inflammatory factors, such as IL-1β, IL-6 and S100β, in the plasma of mice and levels of ADMA, eNOS in tissue lysate were measured with Elisa kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, People's Republic of China), according to the manufacturers' directions. OD at 450 nm was measured with a microplate reader. A standard curve was generated by plotting OD values versus standard concentration. The curve equation was calculated and used to determine the concentrations of samples.

2.5. Evan's blue test

Evan's blue test was done as previously described (Tong et al., 2018). 2 % Evans blue (2 ml/kg) was injected intravenously 30 min before the mice were sacrificed. After the abdominal injection of 2 % pentobarbital sodium (1.5 ml/kg), the chest was opened, the right atrium was cut, and intrahepatic perfusion of heparin saline (0.9 % sodium chloride 20 U/ml heparin sodium) was performed followed by 100 ml rinse. The brain tissue was obtained and frozen section, then stained with 4′,6-diamidino-2-phenylindole (DAPI). The stained sections were observed and photographed under a fluorescence microscope (Olympus, Japan).

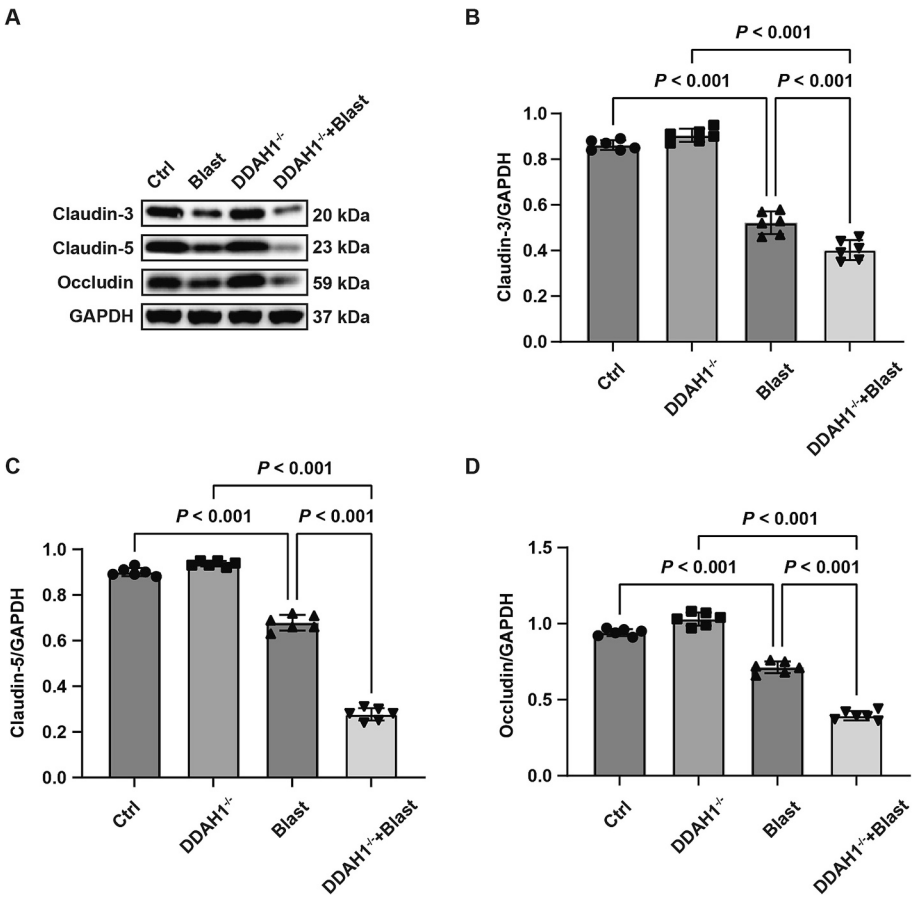

Fig. 3. Expression of tight junction related proteins after thoracic blast exposure.
(A) Western blot of tight junction related proteins. (B) Relative density of Claudin-3. (C) Relative density of Claudin-5. (D) Relative density of Occludin. Data are mean ± SD. $n = 6$ for each group in each experiment.

2.6. Histological analysis

The fixed brain was embedded in paraffin blocks using a Leica Microsystem tissue processor (ASP 300S, Germany). For histological staining, sections of 4 μm thickness were sliced using a Leica Microsystem microtome (Model RM 2265, Germany), which were stained with hematoxylin and eosin (H&E).

2.7. Reactive oxygen species (ROS) detection

Brain tissue sections were stained with 2,3-Dimethoxy-1,4-naphthoquinone (1:100; cat. no. D5439; Sigma, USA) for twenty minutes and then observed and photographed under a fluorescence microscope.

2.8. Western blotting

Western blotting was performed as previously described (Tong et al., 2021). Briefly, brain tissues were lysed and the protein concentrations of tissue homogenates were measured. Then equal amounts of soluble protein were separated on polyacrylamide gels, transferred onto a polyvinylidene fluoride membrane, and followed by western blot analysis. After blocking in 5 % skim milk PBST buffer at room temperature for 1 h, the appropriate primary antibody (Supplementary Table 1) was added and incubated overnight at 4 °C. Then washed 3 times with PBST, and a horseradish peroxidase-labeled secondary antibody (Supplementary Table 2) was incubated for 2 h at room temperature, and then washed for 3 times. Proteins were visualized using a Clarity Western enhanced chemiluminescence Substrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and a ChampChemi 610 Full automatic chemiluminescence image analysis system (Beijing Sage Creation Technology Co., Ltd., Beijing, China).

2.9. Statistical analysis

Data were expressed as the mean ± standard deviation and analyzed using Graphpaad prism 9.5.1. Measurement data were analyzed using one-way ANOVA analysis of variance. A value of $P < 0.05$ was regarded as significant.

3. Results

3.1. DDAH1 knockout exacerbated inflammation and BBB damage in thoracic blast-induced brain injury

As shown in Fig. 1A, inflammatory infiltration of brain tissue was witnessed in blast group of WT mice, which was obviously aggravated in DDAH1$^{-/-}$ mice after thoracic blast exposure. Evans blue test showed that DDAH1 knockout aggravated BBB leakage after thoracic blast exposure ($P < 0.05$, Fig. 1B and C). The levels of IL-1β, IL-6 and S100β in serum were 30.05 ± 3.05 ng/L, 23.98 ± 2.42 pg/ml and 46.25 ± 3.17 pg/ml in WT mice after thoracic blast exposure, separately. It is significantly different comparing with 13.89 ± 1.33 ng/L, 11.88 ± 1.76 pg/ml and 26.68 ± 2.75 pg/ml in control group,. In DDAH1$^{-/-}$ mice, the increase of IL-1β, IL-6 and S100β levels were more significantly, which changes were 14.73 ± 1.32 ng/L to 33.69 ± 1.82 ng/L, 11.28 ± 1.41 pg/ml to 28.46 ± 2.67 pg/ml and 28.34 ± 2.98 pg/ml to 57.43 ± 3.62 pg/ml, separately ($P < 0.05$, Fig. 1D-F).

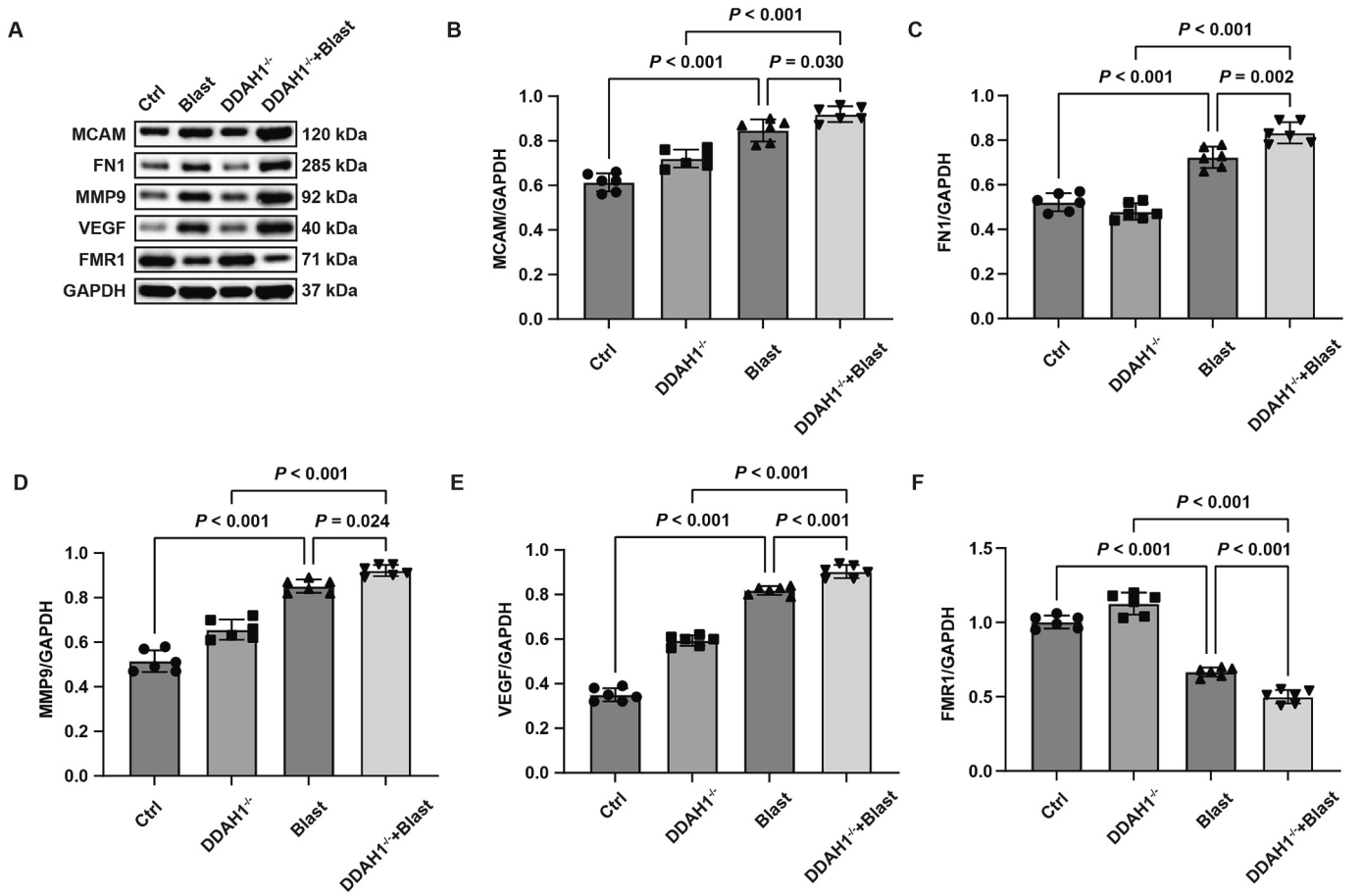

Fig. 4. Changes of vascular endothelial injury related proteins after thoracic blast exposure.
(A) Western blot of vascular endothelial injury related proteins. (B) Relative density of MCAM. (C) Relative density of FN1. (D) Relative density of MMP9. (E) Relative density of VEGF. (F) Relative density of FMR1. Data are mean ± SD. $n = 6$ for each group in each experiment.

3.2. DDAH1 knockout aggravated ROS generation and oxidative stress-related protein changes in the brain tissue after thoracic blast exposure

ROS generation and expressions of IREα and MDA5 were used to evaluate oxidative stress in thoracic blast-induced brain injury. The generation of ROS was detected by 2,3-dimethoxy-1,4-naphthoquinone staining, which was shown as increase of red fluorescence. Our data demonstrated that generation of ROS, MDA5 and IREα expressions clearly increased in WT mice after thoracic blast exposure, whereas DDAH1 knockout significantly aggravated the changes of these factors (Fig. 2, $P < 0.05$).

3.3. DDAH1 knockout aggravated BBB damage after thoracic blast exposure

The expressions of Claudin-3, Claudin-5 and Occludin decreased in brain after thoracic blast exposure, which were less in Blast group of DDAH1$^{-/-}$ mice. The reduction of above tight junction-related proteins indicated lack of DDAH1 would exacerbate BBB damage (Fig. 3).

3.4. DDAH1 knockout aggravated vascular endothelial injury related proteins in the brain tissue after thoracic blast exposure

Angiogenic related proteins are often revolved in vascular endothelial injury. Our results demonstrated that the expressions of MCAM, FN1, MMP9 and VEGF in brain tissue increased after thoracic blast exposure, while the expression of FMR1 decreased ($P < 0.05$). Comparing with WT mice, the increase in MCAM, FN1, MMP9, VEGF levels and the decrease in FMR1 level were more significant in the brain tissue of DDAH1$^{-/-}$ mice after thoracic blast exposure ($P < 0.05$, Fig. 4).

3.5. DDAH1 knockout aggravated changes of focal adhesion related proteins in the brain tissue after thoracic blast exposure

Alteration of focal adhesion (FA) related proteins could affect the function of vascular endothelium. Our results of FA related proteins showed that the levels of p-VASP, Lyn, and LIMA1 in brain tissue decreased in WT mice after thoracic blast exposure, while the level of LIMK1 increased ($P < 0.05$). DDAH1 knockout significantly aggravated the decrease of p-VASP, Lyn, LIMA1 expression and increase of LIMK1 expression in brain tissue after thoracic blast exposure ($P < 0.05$, Fig. 5).

3.6. DDAH1 knockout aggravated dysfunction of cerebral vascular endothelial barrier after thoracic blast exposure

Western blotting analysis showed that Glrb, Sez6, and Dystrophin levels decreased and Vimentin level increased in Blast group comparing with control group. DDAH1 knockout significantly aggravated decreased Glrb, Sez6, and Dystrophin, and increased Vimentin after thoracic blast exposure ($P < 0.05$, Fig. 6).

3.7. DDAH1 knockout aggravated thoracic blast-induced changing in DDAH1/ADMA/eNOS signaling pathway

DDAH1/ADMA/eNOS signaling pathway is important for vascular injury repair. Thoracic blast-induced obvious reduction on DDAH1 and

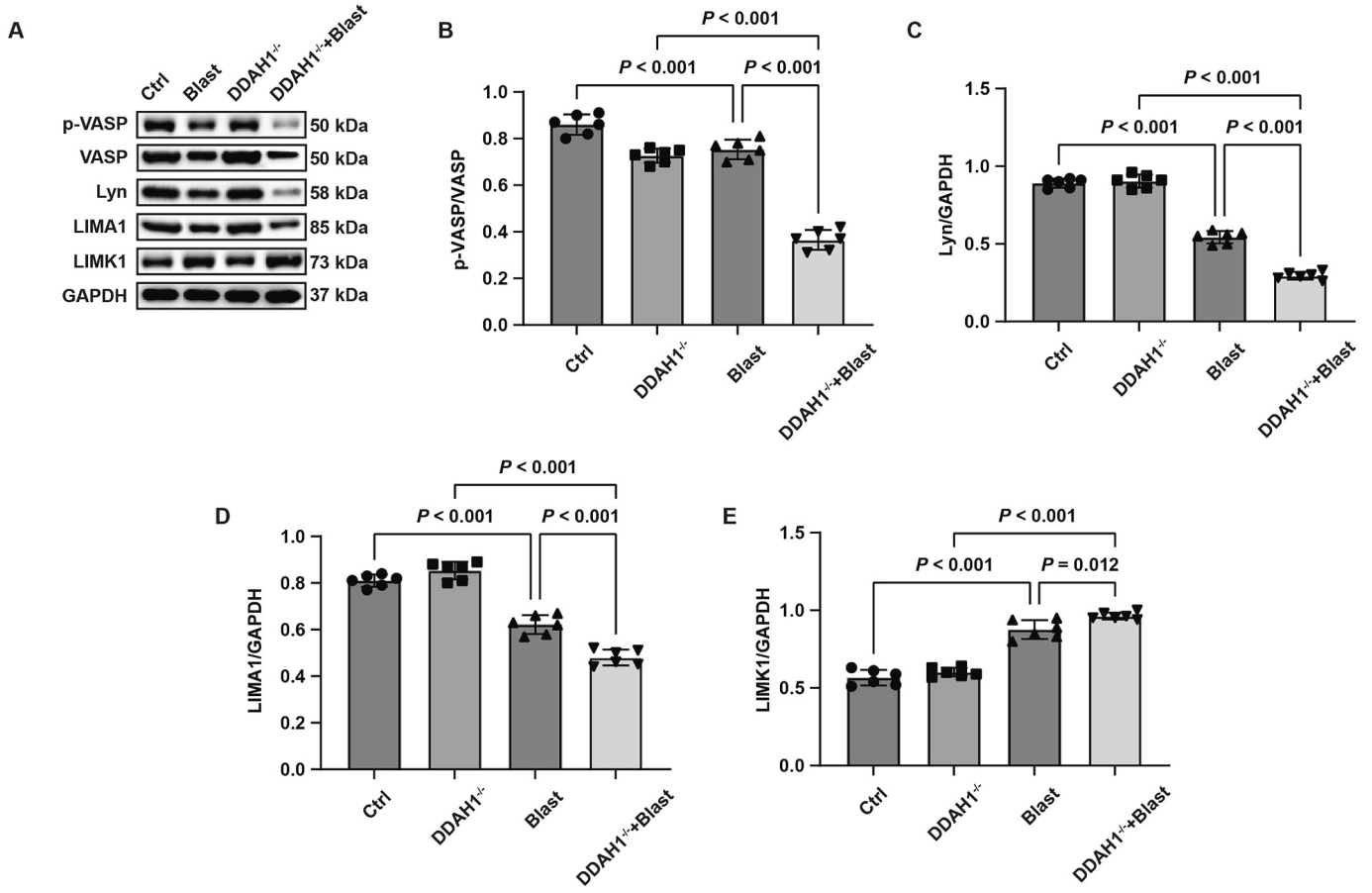

Fig. 5. Expression of focal adhesion related proteins after thoracic blast exposure.
(A) Western blot of focal adhesion related proteins. (B) Relative density of phosphorylation VASP. (C) Relative density of Lyn. (D) Relative density of LIMA1. (E) Relative density of LIMK1. Data are mean ± SD. $n = 6$ for each group in each experiment.

eNOS expression and a significant increase of ADMA content in the brain tissue, while increased ADMA and decreased eNOS were exacerbated in thoracic blast-induced DDAH1$^{-/-}$ mice (Fig. 7A-E, $P < 0.05$). NO content in serum was also decrease due to thoracic blast exposure, and less in blast group of DDAH1$^{-/-}$ mice (Fig. 7F, $P < 0.05$). Upper results demonstrated that DDAH1/ADMA/eNOS was down-regulated by thoracic blast exposure, consequently affected NO content and vascular endothelial injury.

4. Discussion

In this study, we elucidate several critical findings related to thoracic blast exposure: (i) induction of brain injury, evidenced by inflammatory infiltration and elevated serum inflammatory factors, (ii) disruption of BBB, as indicated by increased permeability of BBB and reduction of TJ related proteins; (iii) activation of an oxidative stress responses, as reflected by elevated ROS generation and increased expression of oxidant enzymes; (iv) endothelial vascular injury, characterized by alterations in proteins associated with endothelial barrier dysfunction, FA, and angiogenesis; (v) inhibition of DDAH1 and consequent accumulation of ADMA, leading to decreased eNOS and NO levels; and (vi) aggravation of these effects by DDAH1 deficiency. Collectively, these findings indicate that thoracic blast exposure induces BBB damage and cerebral endothelial barrier dysfunction through the DDHA1/ADMA/eNOS signaling pathway.

Thoracic blast-induced brain injury is a multifaceted condition involving complex mechanisms, such as inflammation, oxidative stress, and apoptosis. Prior studies have demonstrated that serum inflammatory factors could damage distant organs by disrupting the vascular endothelial barrier (Lo-Cao et al., 2021). During this pathological process, cytokine-activated endothelial cells released adhesion molecules, facilitating the transendothelial migration of immune cells and contributing to endothelial dysfunction (*Proteomic Analysis Revealed the Characteristics of Key Proteins Involved in the Regulation of Inflammatory Response, Leukocyte Transendothelial Migration, Phagocytosis, and Immune Process during Early Lung Blast Injury - PubMed*, 2024). Moreover, pro-inflammatory factors exacerbate BBB damage by increasing the influx of harmful substances into the brain, which can lead to cerebrovascular disease (Oikawa et al., 2019). Our previous work has shown that targeting inflammation could alleviate BBB injury, reduce inflammatory infiltration and decrease the expressions of inflammatory proteins in brain tissue (Cong et al., 2019). Thus, we proposed that BBB disruption following thoracic blast exposure was closely associated with the release of inflammatory factors released by BLI. S100β, a biomarker of brain injury, is linked to cerebrovascular diseases (Harpaz et al., 2021). BBB disruption is a crucial factor in the pathogenesis and severity of cerebrovascular conditions (*Dual Microglia Effects on Blood Brain Barrier Permeability Induced by Systemic Inflammation - PubMed*, 2024). Occludin, a 65 kDa transmembrane protein, contributes to tight junction (TJ) stability and BBB function. Degradation of Occludin by vascular risk factors such as ROS, MMPs, Rho-Kinase and VEGF exacerbates BBB disruption (Heyburn et al., 2019). Jiang et al. demonstrated that craniocerebral injury lead to significant BBB damage, which characterized by structural impairments and decreased levels of zonal occludens-1 (ZO-1), Occludin, and Claudin-5 in brain microvascular, alongside increased expression of caspase-3 and vascular injury-related proteins MMP3 and MMP9 (*Phillyrin Prevents Neuroinflammation-Induced Blood-Brain Barrier Damage Following Traumatic Brain Injury via Altering*

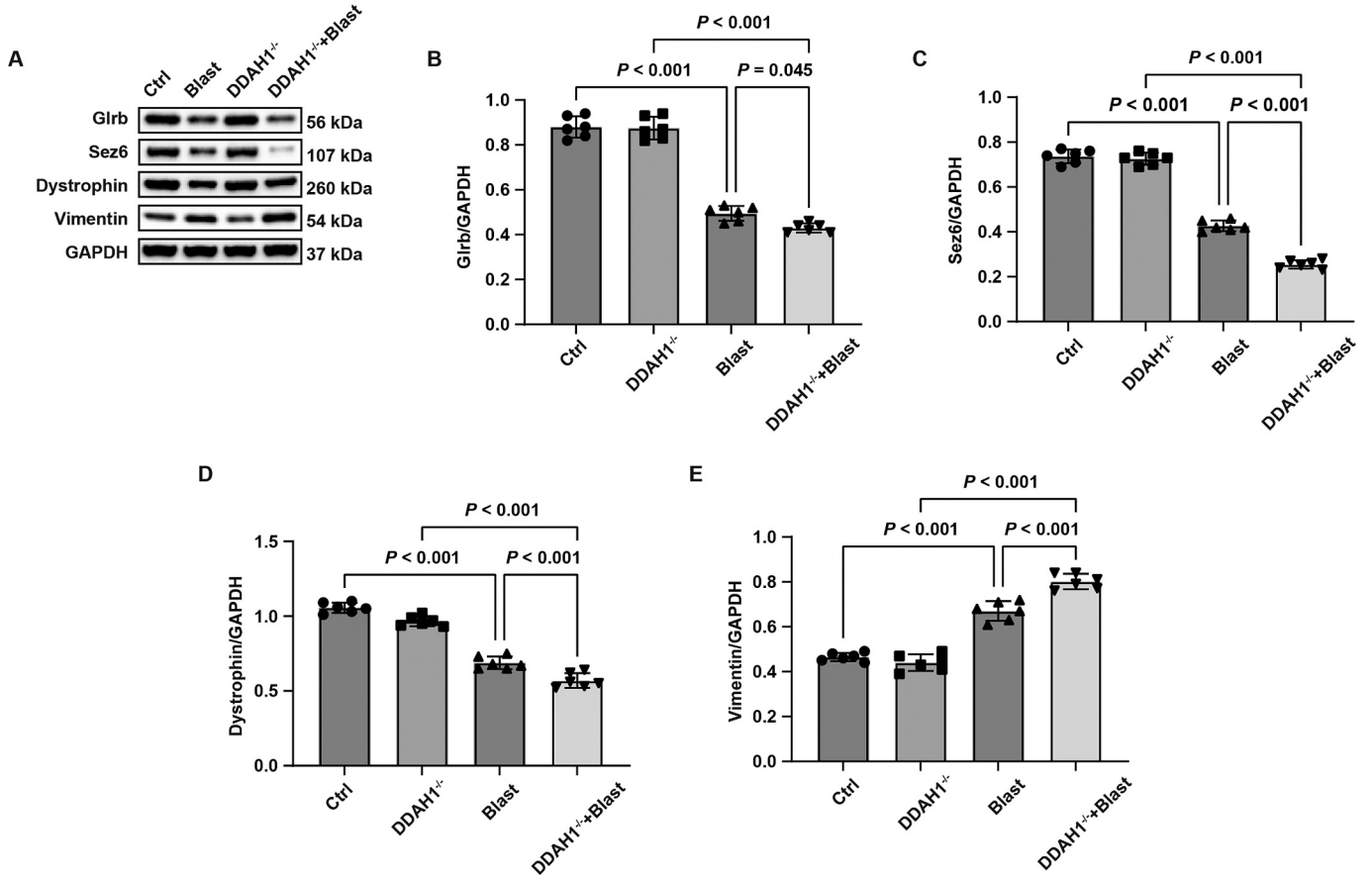

Fig. 6. Changes of cerebral vascular endothelial barrier function-related proteins in the brain tissue after thoracic blast exposure.
(A) Western blot of cerebral vascular endothelial barrier function-related proteins. (B) Relative density of Glrb. (C) Relative density of Sez6. (D) Relative density of Dystrophin. (E) Relative density of Vimentin. Data are mean ± SD. $n = 6$ for each group in each experiment.

Microglial Polarization - PubMed, 2024). Claudin, a main component of TJ, is distributed throughout various organs and is crucial for barrier function (Nakamura et al., 2019). Koichiro et al. found that BBB leakage in neurological and psychiatric disorders was likely due to reactive microglia-induced downregulation of TJ proteins such as Claudin-5, Occludin, and ZO-1. Winkler et al. observed decreased levels of Claudin-1, Claudin-3 and Occludin in brain tissue at 3 h after cerebral ischemia-reperfusion (*Tight Junctions in the Blood-Brain Barrier Promote Edema Formation and Infarct Size in Stroke - Ambivalent Effects of Sealing Proteins - PubMed*, 2024). Wan et al. found that upregulation of ZO-1, Occludin and Claudin-3 could protect BBB permeability in rats with subarachnoid hemorrhage-induced early brain injury (Wan et al., 2021). In endothelial cell-cell junction, TJ, adherent junction(AJ), and FA collectively maintain endothelial integrity to maintain BBB permeability (Zuidema et al., 2020). As a member of the Src non-receptor tyrosine kinases family, Lyn could enhance AJ in regulating endothelial junctions, thus restraining increases in vascular permeability. Lyn knockout mice exhibited increased mortality in LPS-induced endotoxemia and heightened vascular permeability in response to LPS or VEGF challenge compared to wild-type mice. LIMA1, an actin-binding protein, regulates the actin cytoskeleton, stability and extension of AJ, and multiple associations at epithelial cell junctions. Adeline et al. observed deficits between cell-cell junctions and F-actin in LIMA1-silenced HUVECs (Chervin-Pétinot et al., 2012). LIMK1, a kind of serine/threonine protein kinases critical for actin regulation, is primarily localized in neuronal tissue and FA, promoting F-actin assembly (Chen et al., 2021). Skaria et al. found that inhibition of LIMK1 could alleviate Wnt5A-mediated actin cytoskeleton remodeling, AJ disruption, vascular leakage, and barrier dysfunction in human coronary artery endothelial cells (Skaria et al., 2017). Lyn could regulate expression of LIMK1. Park and Kim found that activation of the Lyn-p110β-Rho-associated kinases 1/2 in SKOV3 cells could affect LIMK1 phosphorylation and TGF-β1 release (*PI3K Catalytic Isoform Alteration Promotes the LIMK1-related Metastasis Through the PAK1 or ROCK1/2 Activation in Cigarette Smoke-exposed Ovarian Cancer Cells - PubMed*, 2024). VASP, enriched in focal contacts and stress fibers, is involved in actin-based structures and remodeling, impacting barrier function (Arthur et al., 2021). Decreased VASP phosphorylation was observed in subarachnoid hemorrhage-induced BBB injury (Zhang et al., 2020). Yan et al. found that Tanshinone IIA stimulated VASP phosphorylation to attenuate oxidative stress in endothelial cell (Yan et al., 2021). Collectively, risk factors such as inflammation and ROS induced by thoracic blast exposure diminished the expressions of TJ, AJ and FA-related proteins, leading to BBB leakage and brain injury.

VEGF contributes to BBB disruption and increased central nervous system (CNS) vulnerability by activating MMP-9, which is associated with vascular permeability, TJ proteins degradation, and inflammatory cascades (Shan et al., 2019). MMP9 blockade mitigates vascular damage in spine cord injury and traumatic brain injury. MMP9 also regulates extracellular matrix components, laminin, TJ-related proteins. Blockade of MMPs during the initial injury phase reduces vascular damage and improves locomotors recovery (*VEGF Regulates the Blood-Brain Barrier through MMP-9 in a Rat Model of Traumatic Brain Injury - PubMed*, 2024). Elevated VEGF and MMP9 levels are commonly observed in traumatic brain injury, and their inhibition appears to improve BBB function. As a VEGF co-receptor, MCAM is predominantly expressed at the intercellular junctions of endothelial cells to mediate the interaction between endothelial cells and pericytes, thus promoting BBB development

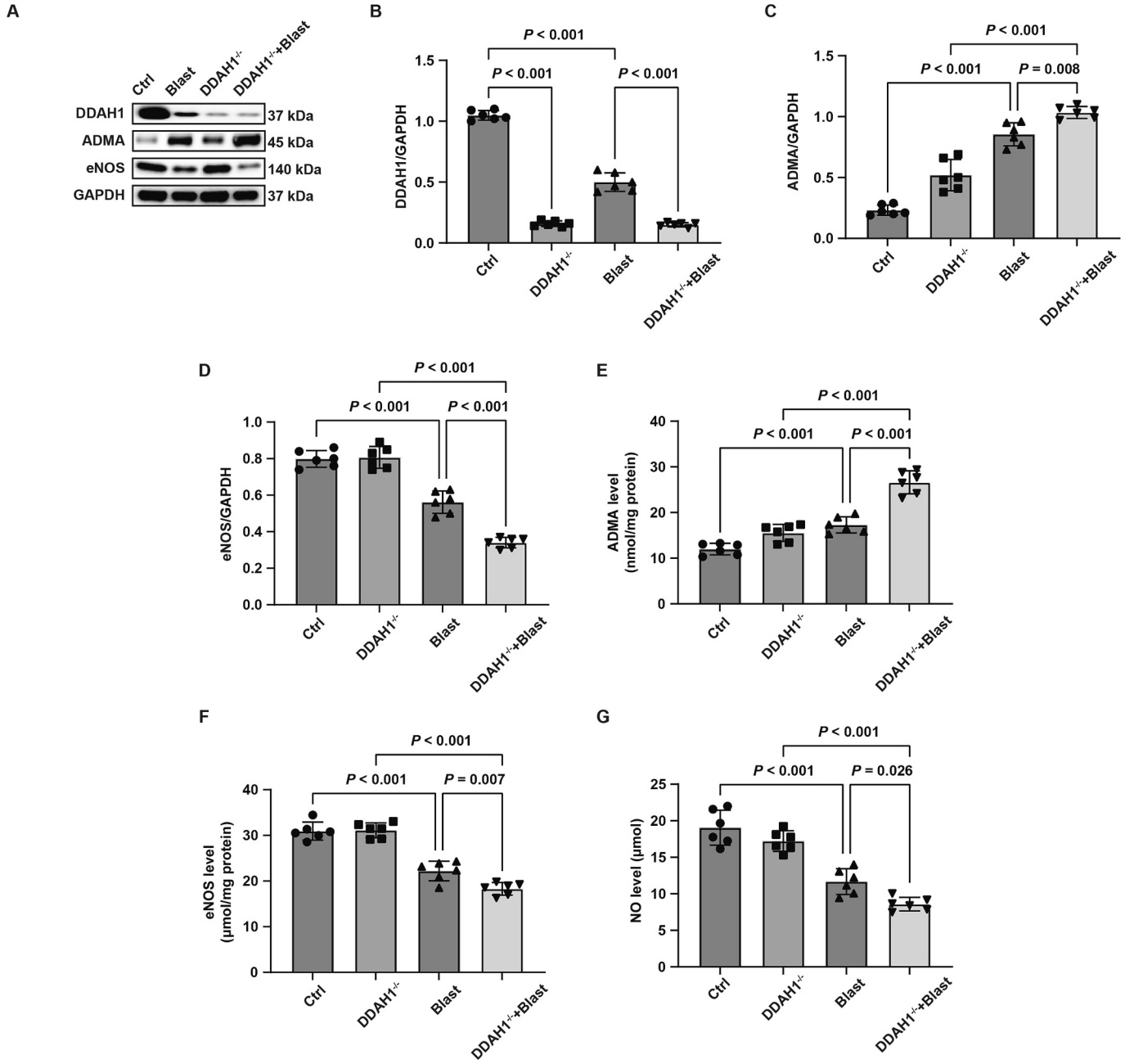

Fig. 7. Changes of DDAH1/ADMA/eNOS signal pathway after thoracic blast exposure.
(A) Western blot of DDAH1/ADMA/eNOS signaling pathway in each group. (B) Relative density of DDAH1. (C) Relative density of ADMA. (D) Relative density of eNOS. (E) ADMA level in brain tissue. (F) eNOS level in brain tissue. (G) NO content in serum. Data are mean ± SD. $n = 6$ for each group in each experiment.

(*Targeting CD146 using Folic Acid-Conjugated Nanoparticles and Suppression of Tumor Growth in a Mouse Glioma Model - PubMed*, 2024). Elevated MCAM levels in BBB endothelial cells under neuroinflammatory conditions facilitate inflammatory cell transmigration into the CNS (Zondler et al., 2020). FN1, a major ECM component, plays a key role in cell adhesion, growth, migration, and differentiation. Wang et al. reported increased FN1 levels in severe brain edema, hemorrhagic transformation, and poor functional outcome. They suggested that high levels of MMP9 and FN1 may indicate severe neurovascular damage (*Association of Matrix Metalloproteinase 9 and Cellular Fibronectin and Outcome in Acute Ischemic Stroke: A Systematic Review and Meta-Analysis - PubMed*, 2024). Vimentin contributes to pathogen invasion, neutrophil recruitment, BBB permeability, and neuronal inflammation. In Patrick's study, vimentin increased significantly at 24 h after mild TBI in rats (*Screening of Biochemical and Molecular Mechanisms of Secondary Injury and Repair in the Brain after Experimental Blast-Induced Traumatic Brain Injury in Rats - PubMed*, 2024). A proteomic study confirmed that glial fibrillary acidic protein and vimentin were affected by traumatic brain injury for over two weeks in pigs (Attilio et al., 2017). Sez6, a transmembrane protein associated with neurodevelopment and mental disorders, impairs motor learning, short-term spatial memory and working memory in knockout mice (*Lack of Sez6 Family Proteins Impairs Motor Functions, Short-Term Memory, and Cognitive Flexibility and Alters Dendritic Spine Properties - PubMed*, 2024). Zhu et al. found that β - amyloid protein could impair synaptic plasticity by inhibiting Sez6 (*Beta-Site Amyloid Precursor Protein Cleaving Enzyme 1 Inhibition Impairs Synaptic Plasticity via Seizure Protein 6 - PubMed*, 2024). Glrb, a major inhibitory neurotransmitter receptor, is involved in brain's defensive response.

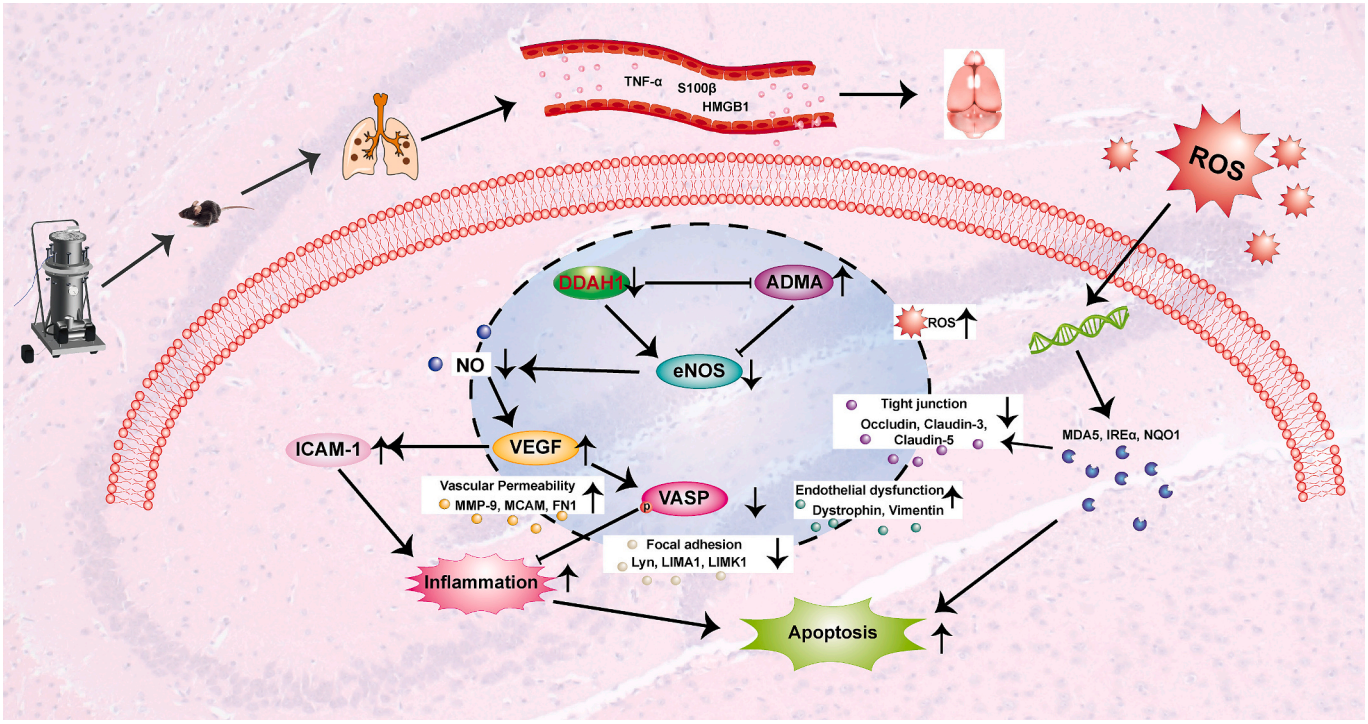

Fig. 8. DDAH1 deficiency exacerbates cerebral vascular endothelial dysfunction by aggravating BBB disruption and oxidative stress in thoracic blast-induced brain injury.

Damage to Glrb increased susceptibility to synaptic dysfunction-related diseases such as epilepsy, autism, panic disorder, and Parkinson's disease (*Glycine is a Competitive Antagonist of the TNF Receptor Mediating the Expression of Inflammatory Cytokines in 3T3-L1 Adipocytes - PubMed*, 2024; *GLRB Allelic Variation Associated with Agoraphobic Cognitions, Increased Startle Response and Fear Network Activation: A Potential Neurogenetic Pathway to Panic Disorder - PubMed*, 2024). Dystrophin, involved in synaptic transmission and neuroglial angiogenesis in the BBB, is associated with cerebral vascular dysfunction when knocked out, leading to cerebral edema, BBB leakage, and decreased levels of TJ-related proteins. Caudal et al. found that low Dystrophin level is linked to attention deficit, memory impairment, and increased risk of epilepsy (*Characterization of Brain Dystrophins Absence and Impact in Dystrophin-Deficient Dmdmdx Rat Model - PubMed*, 2024). These findings collectively suggested that endothelial dysfunction is a key feature of brain injury following thoracic blast exposure.

DDAH1, a crucial regulator of NO production, promotes vascular endothelial repair and maintains physiological NO levels essential for vascular integrity. Increasing ADMA or other stresses could impair DDAH1 activity, leading to endothelial dysfunction (Yang et al., 2018; Wang et al., 2020). As a principal NOS subtype regulating vascular function, eNOS counteracts TJ protein degradation to alleviate BBB injury. Zhao et al. demonstrated that DDAH1 could protect TJ proteins to maintain BBB permeability by reducing ADMA level and increasing NO production (Zhao et al., 2021). Pan et al. found that miR-132-3p could up-regulate eNOS level to relieve ROS generation, TJ proteins reduction, and apoptosis of endothelial cells in cerebral ischemia-reperfusion injury (Pan et al., 2020). In a cerebrovascular disease' s study, Zhou et al. observed that β-amyloid protein-induced attenuation of eNOS activity lead to chronic reduction in cerebral blood flow, BBB leakage, TJ proteins reduction, endothelial dysfunction, microbleeds, Tau hyperphosphorylation, synaptic loss, and cognitive impairment (Zhou et al., 2022). Beyond regulation of TJ proteins, the DDAH1/ADMA/eNOS signaling pathway could reduce VASP phosphorylation to affect endothelial cell polarization and FA stability, leading to TJ integrity disruption and endothelial dysfunction (Wojciak-Stothard et al., 2009). Our study demonstrates that DDAH1 knockout aggravated TJ proteins reduction and FA instability following thoracic blast exposure, underscoring the essential role of DDAH1 in maintaining BBB integrity by preserving TJ and AJ stability.

The DDAH1/ADMA/eNOS signaling pathway is integral to the regulation of vascular endothelial barrier function. Garbincius et al. found that overexpression of DDAH1 could promote NO signaling and partially restore dystrophin expression in patients with duchenne muscular dystrophy (Garbincius et al., 2020). The synthesis of NO and VEGF levels is essential for vascular repair and angiogenesis (Daly et al., 2017). Activation of the eNOS-VEGF pathway could improve angiogenesis by promoting endothelial cell survival, migration, and tubular formation. An et al. observed that bilateral carotid artery stenosis resulted in exacerbated vascular injury in eNOS knockout mice, manifesting as increased white matter and axonal damage, augmented BBB leakage, elevated inflammatory responses, and worsened cognitive deficits (An et al., 2021). Furthermore, elevated eNOS level have been linked to neuroprotective effects of blood vessels in ischemic stroke, mitigating BBB leakage, TJ protein degradation, MMP9 activity, oxidative stress and neutrophil infiltration (Yang et al., 2021). Liu et al. reported that DDAH1 downregulated α-SMA, collagen I, and FN1 while upregulating E-cadherin (Liu et al., 2016). Lv et al. also revealed that increased eNOS and NO production in endothelial cells could improve endothelial function by reducing MCAM expression (Lv et al., 2017). In our study, DDAH1 knockout exacerbated endothelial dysfunction after thoracic blast exposure.

Based on these observations, we propose the relevant mechanism of thoracic blast exposure-induced brain injury, as illustrated in Fig. 8. Thoracic blast exposure triggers the release of inflammatory factors into the serum, resulting in BBB disruption, oxidative stress responses, and cerebral vascular endothelial dysfunction. DDAH1 crucially regulates this process by maintaining NO content through DDAH1/ADMA/eNOS signaling pathway. Reduced DDAH1 exacerbates alterations in proteins associated with TJ damage, FA disruption, and endothelial dysfunction, which in turn aggravates brain injury.

5. Conclusion

In summary, we have identified a novel mechanism by which cerebrovascular risk factors contribute to brain injury following thoracic blast exposure. Thoracic blast-induced elevation of serum inflammatory factors the down-regulated DDAH1, leading to the accumulation of ADMA and a consequent reduction in eNOS and NO production. This disruption of the DDAH1/ADMA/eNOS signaling pathway drove the degradation of TJ, AJ, FA proteins, culminating in endothelial dysfunction. These early pathological changes significantly compromise the integrity of the BBB, resulting in pronounced brain injury. Given the crucial role of DDAH1 in mitigating these effects, our findings suggested that DDAH1 supplementation or activation of DDAH1-related signaling pathways may offer promising therapeutic strategies for preventing or managing thoracic blast exposure-associated brain injury complications.

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Author contributions

Yunen Liu contributed to conceptualization, project administration, and supervision. Peifang Cong wrote the manuscript. Mingxiao Hou revised the manuscript. Peifang Cong, Changci Tong, Shun Mao and Lin Shi conducted the experiments and analyzed the data. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Peifang Cong: Visualization, Project administration, Methodology, Conceptualization, Writing – review & editing, Writing – original draft. **Changci Tong:** Validation. **Shun Mao:** Methodology. **Lin Shi:** Investigation. **Mingxiao Hou:** Resources. **Yunen Liu:** Supervision, Project administration, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Almazroue, H., Jin, Y., Nelin, L.D., Barba, J.C., Milton, A.D., Trittmann, J.K., 2023. Human pulmonary microvascular endothelial cell DDAH1-mediated nitric oxide production promotes pulmonary smooth muscle cell apoptosis in co-culture. Am. J. Phys. Lung Cell. Mol. Phys. 325, L360–L367. https://doi.org/10.1152/ajplung.00433.2021.

An, L., Shen, Y., Chopp, M., Zacharek, A., Venkat, P., Chen, Z., Li, W., Qian, Y., Landschoot-Ward, J., Chen, J., 2021. Deficiency of endothelial nitric oxide synthase (eNOS) exacerbates brain damage and cognitive deficit in a mouse model of vascular dementia. Aging Dis. 12, 732–746. https://doi.org/10.14336/AD.2020.0523.

Arthur, A.L., Crawford, A., Houdusse, A., Titus, M.A., 2021. VASP-mediated actin dynamics activate and recruit a filopodia myosin. eLife 10, e68082. https://doi.org/10.7554/eLife.68082.

Association of Matrix Metalloproteinase 9 and Cellular Fibronectin and Outcome in Acute Ischemic Stroke: A Systematic Review and Meta-Analysis - PubMed. https://pubmed.ncbi.nlm.nih.gov/33329294/, 2024 (accessed May 17, 2024).

Attilio, P.J., Flora, M., Kamnaksh, A., Bradshaw, D.J., Agoston, D., Mueller, G.P., 2017. The effects of blast exposure on protein Deimination in the brain. Oxidative Med. Cell. Longev. 2017, 8398072. https://doi.org/10.1155/2017/8398072.

Beta-Site Amyloid Precursor Protein Cleaving Enzyme 1 Inhibition Impairs Synaptic Plasticity via Seizure Protein 6 - PubMed. https://pubmed.ncbi.nlm.nih.gov/28129943/, 2024 (accessed September 2, 2024).

Characterization of Brain Dystrophins Absence and Impact in Dystrophin-Deficient Dmdmdx Rat Model - PubMed. https://pubmed.ncbi.nlm.nih.gov/32160266/, 2024 (accessed September 2, 2024).

Chen, X., Li, H., Wang, Z., Zhou, Q., Chen, S., Yang, B., Yin, D., He, H., He, M., 2020. Quercetin protects the vascular endothelium against iron overload damages via ROS/ADMA/DDAHII/eNOS/NO pathway. Eur. J. Pharmacol. 868, 172885. https://doi.org/10.1016/j.ejphar.2019.172885.

Chen, P., Jing, H., Xiong, M., Zhang, Q., Lin, D., Ren, D., Wang, S., Yin, D., Chen, Y., Zhou, T., Li, B., Fei, E., Pan, B.-X., 2021. Spine impairment in mice high-expressing neuregulin 1 due to LIMK1 activation. Cell Death Dis. 12, 403. https://doi.org/10.1038/s41419-021-03687-8.

Chervin-Pétinot, A., Courçon, M., Almagro, S., Nicolas, A., Grichine, A., Grunwald, D., Prandini, M.-H., Huber, P., Gulino-Debrac, D., 2012. Epithelial protein lost in neoplasm (EPLIN) interacts with α-catenin and actin filaments in endothelial cells and stabilizes vascular capillary network in vitro. J. Biol. Chem. 287, 7556–7572. https://doi.org/10.1074/jbc.M111.328682.

Chitosan Oligosaccharide Ameliorates Acute Lung Injury Induced by Blast Injury through the DDAH1/ADMA Pathway - PubMed. https://pubmed.ncbi.nlm.nih.gov/29415054/, 2024 (accessed May 17, 2024).

Cong, P., Tong, C., Liu, Y., Shi, L., Shi, X., Zhao, Y., Xiao, K., Jin, H., Liu, Y., Hou, M., 2019. CD28 deficiency ameliorates thoracic blast exposure-induced oxidative stress and apoptosis in the brain through the PI3K/Nrf2/Keap1 signaling pathway. Oxidative Med. Cell. Longev. 2019, 8460290. https://doi.org/10.1155/2019/8460290.

Cong, P., Wang, T., Tong, C., Liu, Y., Shi, L., Mao, S., Shi, X., Jin, H., Liu, Y., Hou, M., 2021. Resveratrol ameliorates thoracic blast exposure-induced inflammation, endoplasmic reticulum stress and apoptosis in the brain through the Nrf2/Keap1 and NF-κB signaling pathway. Injury 52, 2795–2802. https://doi.org/10.1016/j.injury.2021.08.019.

Cong, P., Tong, C., Mao, S., Shi, L., Shi, X., Liu, Y., Jin, H., Liu, Y., Hou, M., 2022. DDAH1 promotes lung endothelial barrier repair by decreasing leukocyte Transendothelial migration and oxidative stress in explosion-induced lung injury. Oxidative Med. Cell. Longev. 2022, 8407635. https://doi.org/10.1155/2022/8407635.

Daly, K.P., Stack, M., Eisenga, M.F., Keane, J.F., Zurakowski, D., Blume, E.D., Briscoe, D. M., 2017. Vascular endothelial growth factor a is associated with the subsequent development of moderate or severe cardiac allograft vasculopathy in pediatric heart transplant recipients. J. Heart Lung Transplant. Off. Publ. Int. Soc. Heart Transplant. 36, 434–442. https://doi.org/10.1016/j.healun.2016.09.013.

Dual Microglia Effects on Blood Brain Barrier Permeability Induced by Systemic Inflammation - PubMed. https://pubmed.ncbi.nlm.nih.gov/31862977/, 2024 (accessed May 17, 2024).

Garbincius, J.F., Merz, L.E., Cuttitta, A.J., Bayne, K.V., Schrade, S., Armstead, E.A., Converso-Baran, K.L., Whitesall, S.E., D'Alecy, L.G., Michele, D.E., 2020. Enhanced dimethylarginine degradation improves coronary flow reserve and exercise tolerance in Duchenne muscular dystrophy carrier mice. Am. J. Physiol. Heart Circ. Physiol. 319, H582–H603. https://doi.org/10.1152/ajpheart.00333.2019.

GLRB Allelic Variation Associated with Agoraphobic Cognitions, Increased Startle Response and Fear Network Activation: A Potential Neurogenetic Pathway to Panic Disorder - PubMed. https://pubmed.ncbi.nlm.nih.gov/28167838/, 2024 (accessed September 2, 2024).

Glycine is a Competitive Antagonist of the TNF Receptor Mediating the Expression of Inflammatory Cytokines in 3T3-L1 Adipocytes - PubMed. https://pubmed.ncbi.nlm.nih.gov/33877377/, 2024 (accessed September 2, 2024).

Harpaz, D., Bajpai, R., Ng, G.J.L., Soljak, M., Marks, R.S., Cheung, C., Arumugam, T.V., Quek, A.M.L., Tok, A.I.Y., Seet, R.C.S., 2021. Blood biomarkers to detect new-onset atrial fibrillation and cardioembolism in ischemic stroke patients. Heart Rhythm. 18, 855–861. https://doi.org/10.1016/j.hrthm.2021.01.032.

Heyburn, L., Abutarboush, R., Goodrich, S., Urioste, R., Batuure, A., Statz, J., Wilder, D., Ahlers, S.T., Long, J.B., Sajja, V.S.S.S., 2019. Repeated low-level blast overpressure leads to endovascular disruption and alterations in TDP-43 and Piezo2 in a rat model of blast TBI. Front. Neurol. 10, 766. https://doi.org/10.3389/fneur.2019.00766.

Lack of Sez6 Family Proteins Impairs Motor Functions, Short-Term Memory, and Cognitive Flexibility and Alters Dendritic Spine Properties - PubMed. https://pubmed.ncbi.nlm.nih.gov/31711114/, 2024 (accessed September 2, 2024).

Li, N., Geng, C., Hou, S., Fan, H., Gong, Y., 2020. Damage-associated molecular patterns and their signaling pathways in primary blast lung injury: new research Progress and future directions. Int. J. Mol. Sci. 21, 6303. https://doi.org/10.3390/ijms21176303.

Liu, X.-J., Hong, Q., Wang, Z., Yu, Y., Zou, X., Xu, L., 2016. MicroRNA21 promotes interstitial fibrosis via targeting DDAH1: a potential role in renal fibrosis. Mol. Cell. Biochem. 411, 181–189. https://doi.org/10.1007/s11010-015-2580-2.

Liu, Y., Feng, K., Jiang, H., Hu, F., Gao, J., Zhang, W., Zhang, W., Huang, B., Brant, R., Zhang, C., Yan, H., 2020. Characteristics and treatments of ocular blast injury in Tianjin explosion in China. BMC Ophthalmol. 20, 185. https://doi.org/10.1186/s12886-020-01448-3.

Lo-Cao, E., Hall, S., Parsell, R., Dandie, G., Fahlström, A., 2021. Neurogenic pulmonary edema. Am. J. Emerg. Med. 45 (678), e3–678.e5. https://doi.org/10.1016/j.ajem.2020.11.052.

Lv, L., Qi, H., Guo, X., Ni, Q., Yan, Z., Zhang, L., 2017. Long noncoding RNA uc001pwg.1 is downregulated in Neointima in arteriovenous fistulas and mediates the function of endothelial cells derived from pluripotent stem cells. Stem Cells Int. 2017, 4252974. https://doi.org/10.1155/2017/4252974.

Nakamura, S., Irie, K., Tanaka, H., Nishikawa, K., Suzuki, H., Saitoh, Y., Tamura, A., Tsukita, S., Fujiyoshi, Y., 2019. Morphologic determinant of tight junctions revealed

by claudin-3 structures. Nat. Commun. 10, 816. https://doi.org/10.1038/s41467-019-08760-7.
Oikawa, S., Kai, Y., Mano, A., Sugama, S., Mizoguchi, N., Tsuda, M., Muramoto, K., Kakinuma, Y., 2019. Potentiating a non-neuronal cardiac cholinergic system reinforces the functional integrity of the blood brain barrier associated with systemic anti-inflammatory responses. Brain Behav. Immun. 81, 122–137. https://doi.org/10.1016/j.bbi.2019.06.005.
Pan, Q., Kuang, X., Cai, S., Wang, X., Du, D., Wang, J., Wang, Y., Chen, Y., Bihl, J., Chen, Y., Zhao, B., Ma, X., 2020. miR-132-3p priming enhances the effects of mesenchymal stromal cell-derived exosomes on ameliorating brain ischemic injury. Stem Cell Res Ther 11, 260. https://doi.org/10.1186/s13287-020-01761-0.
Pearce, A.P., Bull, A.M.J., Clasper, J.C., 2017. Mediastinal injury is the strongest predictor of mortality in mounted blast amongst UK deployed forces. Injury 48, 1900–1905. https://doi.org/10.1016/j.injury.2017.07.019.
Phillyrin Prevents Neuroinflammation-Induced Blood-Brain Barrier Damage Following Traumatic Brain Injury via Altering Microglial Polarization - PubMed. https://pubmed.ncbi.nlm.nih.gov/34744713/, 2024 (accessed May 17, 2024).
Phipps, H., Mondello, S., Wilson, A., Dittmer, T., Rohde, N.N., Schroeder, P.J., Nichols, J., McGirt, C., Hoffman, J., Tanksley, K., Chohan, M., Heiderman, A., Abou Abbass, H., Kobeissy, F., Hinds, S., 2020. Characteristics and impact of U.S. military blast-related mild traumatic brain injury: a systematic review. Front. Neurol. 11, 559318. https://doi.org/10.3389/fneur.2020.559318.
PI3K Catalytic Isoform Alteration Promotes the LIMK1-related Metastasis Through the PAK1 or ROCK1/2 Activation in Cigarette Smoke-exposed Ovarian Cancer Cells - PubMed. https://pubmed.ncbi.nlm.nih.gov/28373445/, 2024 (accessed September 2, 2024).
Proteomic Analysis Revealed the Characteristics of Key Proteins Involved in the Regulation of Inflammatory Response, Leukocyte Transendothelial Migration, Phagocytosis, and Immune Process during Early Lung Blast Injury - PubMed. https://pubmed.ncbi.nlm.nih.gov/34007409/, 2024 (accessed May 17, 2024).
Reddy, K.R.K., Dasari, C., Duscharla, D., Supriya, B., Ram, N.S., Surekha, M.V., Kumar, J. M., Ummanni, R., 2018. Dimethylarginine dimethylaminohydrolase-1 (DDAH1) is frequently upregulated in prostate cancer, and its overexpression conveys tumor growth and angiogenesis by metabolizing asymmetric dimethylarginine (ADMA). Angiogenesis 21, 79–94. https://doi.org/10.1007/s10456-017-9587-0.
Screening of Biochemical and Molecular Mechanisms of Secondary Injury and Repair in the Brain after Experimental Blast-Induced Traumatic Brain Injury in Rats - PubMed. https://pubmed.ncbi.nlm.nih.gov/23496248/, 2024 (accessed September 2, 2024).
Shan, Y., Tan, S., Lin, Y., Liao, S., Zhang, B., Chen, X., Wang, J., Deng, Z., Zeng, Q., Zhang, L., Wang, Y., Hu, X., Qiu, W., Peng, L., Lu, Z., 2019. The glucagon-like peptide-1 receptor agonist reduces inflammation and blood-brain barrier breakdown in an astrocyte-dependent manner in experimental stroke. J. Neuroinflammation 16, 242. https://doi.org/10.1186/s12974-019-1638-6.
Skaria, T., Bachli, E., Schoedon, G., 2017. WIF1 prevents Wnt5A mediated LIMK/CFL phosphorylation and adherens junction disruption in human vascular endothelial cells. J. Inflamm. Lond. Engl. 14, 10. https://doi.org/10.1186/s12950-017-0157-4.
Targeting CD146 using Folic Acid-Conjugated Nanoparticles and Suppression of Tumor Growth in a Mouse Glioma Model - PubMed. https://pubmed.ncbi.nlm.nih.gov/32707539/, 2024 (accessed May 17, 2024).
Tight Junctions in the Blood-Brain Barrier Promote Edema Formation and Infarct Size in Stroke - Ambivalent Effects of Sealing Proteins - PubMed. https://pubmed.ncbi.nlm.nih.gov/32054373/, 2024 (accessed May 17, 2024).
Tong, C., Liu, Y., Zhang, Y., Cong, P., Shi, X., Liu, Y., Jin, L. Shi Hongxu, Hou, M., 2018. Shock waves increase pulmonary vascular leakage, inflammation, oxidative stress, and apoptosis in a mouse model. Exp. Biol. Med. Maywood NJ 243, 934–944. https://doi.org/10.1177/1535370218784539.
Tong, C., Cong, P., Liu, Y., Shi, X., Shi, L., Mao, S., Zhao, Y., Hou, M., Liu, Y., 2021. Tandem mass tag-based quantitative proteomic analysis reveals pathways involved in brain injury induced by chest exposure to shock waves. Front. Mol. Neurosci. 14, 688050. https://doi.org/10.3389/fnmol.2021.688050.
VEGF Regulates the Blood-Brain Barrier through MMP-9 in a Rat Model of Traumatic Brain Injury - PubMed. https://pubmed.ncbi.nlm.nih.gov/36382093/, 2024 (accessed May 17, 2024).
Wan, Y., Song, M., Xie, X., Chen, Z., Gao, Z., Wu, X., Huang, R., Chen, M., 2021. BMSCs regulate astrocytes through TSG-6 to protect the blood-brain barrier after subarachnoid hemorrhage. Mediat. Inflamm. 2021, 5522291. https://doi.org/10.1155/2021/5522291.
Wang, H.-B., Wang, W.-Q., Wu, Q.-J., Hou, Y.-J., Li, H.-X., Yang, H.-J., Yang, M.-F., Sun, B.-L., Zhang, Z.-Y., 2020. Negative allosteric modulator of mGluR1 improves Long-term neurologic deficits after experimental subarachnoid hemorrhage. ACS Chem. Neurosci. 11, 2869–2880. https://doi.org/10.1021/acschemneuro.0c00485.
Wojciak-Stothard, B., Torondel, B., Zhao, L., Renné, T., Leiper, J.M., 2009. Modulation of Rac1 activity by ADMA/DDAH regulates pulmonary endothelial barrier function. Mol. Biol. Cell 20, 33–42. https://doi.org/10.1091/mbc.e08-04-0395.
Wolf, S.J., Bebarta, V.S., Bonnett, C.J., Pons, P.T., Cantrill, S.V., 2009. Blast injuries. Lancet Lond. Engl. 374, 405–415. https://doi.org/10.1016/S0140-6736(09)60257-9.
Yan, Q., Mao, Z., Hong, J., Gao, K., Niimi, M., Mitsui, T., Yao, J., 2021. Tanshinone IIA stimulates cystathionine γ-Lyase expression and protects endothelial cells from oxidative injury. Antioxid. Basel Switz. 10, 1007. https://doi.org/10.3390/antiox10071007.
Yang, D., Tan, S., Yang, Z., Jiang, P., Qin, C., Yuan, Q., Dang, R., Yao, X., Qu, J., Lu, Q., Xu, P., Zhang, B., Xiang, D., Chen, L., 2018. Dihydromyricetin attenuates TNF-α-induced endothelial dysfunction through miR-21-mediated DDAH1/ADMA/NO signal pathway. Biomed. Res. Int. 2018, 1047810. https://doi.org/10.1155/2018/1047810.
Yang, C., Lavayen, B.P., Liu, L., Sanz, B.D., DeMars, K.M., Larochelle, J., Pompilus, M., Febo, M., Sun, Y.-Y., Kuo, Y.-M., Mohamadzadeh, M., Farr, S.A., Kuan, C.-Y., Butler, A.A., Candelario-Jalil, E., 2021. Neurovascular protection by adropin in experimental ischemic stroke through an endothelial nitric oxide synthase-dependent mechanism. Redox Biol. 48, 102197. https://doi.org/10.1016/j.redox.2021.102197.
Zhang, C., Jiang, M., Wang, W.-Q., Zhao, S.-J., Yin, Y.-X., Mi, Q.-J., Yang, M.-F., Song, Y.-Q., Sun, B.-L., Zhang, Z.-Y., 2020. Selective mGluR1 negative allosteric modulator reduces blood-brain barrier permeability and cerebral edema after experimental subarachnoid hemorrhage. Transl. Stroke Res. 11, 799–811. https://doi.org/10.1007/s12975-019-00758-z.
Zhao, Y., Ma, X., Zhou, Y., Xie, J., Liu, X., Zhao, Y., 2021. DDAH-1, via regulation of ADMA levels, protects against ischemia-induced blood-brain barrier leakage. Lab. Investig. J. Tech. Methods Pathol. 101, 808–823. https://doi.org/10.1038/s41374-021-00541-5.
Zhou, H., Gao, F., Yang, X., Lin, T., Li, Z., Wang, Q., Yao, Y., Li, L., Ding, X., Shi, K., Liu, Q., Bao, H., Long, Z., Wu, Z., Vassar, R., Cheng, X., Li, R., Shen, Y., 2022. Endothelial BACE1 impairs cerebral small vessels via tight junctions and eNOS. Circ. Res. 130, 1321–1341. https://doi.org/10.1161/CIRCRESAHA.121.320183.
Zondler, L., Herich, S., Kotte, P., Körner, K., Schneider-Hohendorf, T., Wiendl, H., Schwab, N., Zarbock, A., 2020. MCAM/CD146 signaling via PLCγ1 leads to activation of β1-Integrins in memory T-cells resulting in increased brain infiltration. Front. Immunol. 11, 599936. https://doi.org/10.3389/fimmu.2020.599936.
Zuidema, A., Wang, W., Sonnenberg, A., 2020. Crosstalk between cell adhesion complexes in regulation of Mechanotransduction. BioEssays News Rev. Mol. Cell. Dev. Biol. 42, e2000119. https://doi.org/10.1002/bies.202000119.