

Mechanisms of Blood–Brain Barrier Disruption and Subsequent Cellular Injury in Animal Models of Neurological Disorders

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ABSTRACT

Background: Disruption of the blood–brain barrier (BBB) is a critical event in the pathogenesis of neurological disorders, contributing to the progression of cellular injury and neurological dysfunction. Experimental animal models provide valuable insights into the molecular and cellular mechanisms underlying BBB breakdown and its downstream effects.

Objective: This study aimed to investigate the mechanisms of BBB disruption and subsequent cellular injury in animal models of neurological disorders, with a focus on the roles of tight junction degradation, inflammation, oxidative stress, and apoptosis.

Methods: Experimental models of ischemic stroke (middle cerebral artery occlusion), traumatic brain injury (controlled cortical impact), and neuroinflammation (lipopolysaccharide administration) were established in rodents. BBB integrity was assessed using Evans Blue extravasation and fluorescent tracer permeability assays. Tight junction protein expression (occluding, claudin-5, ZO-1) was evaluated by immunohistochemistry and Western blotting. Inflammatory mediators, oxidative stress markers, and apoptotic pathways were analyzed using ELISA, biochemical assays, qRT-PCR, and protein expression profiling. Histopathological evaluation and behavioral assessments were conducted to determine the extent of neuronal injury and functional impairment.

Results: All injury models demonstrated significant BBB disruption, characterized by increased vascular permeability and loss of tight junction integrity. Upregulation of matrix metalloproteinases (MMP-2 and MMP-9), pro-inflammatory cytokines (TNF- α , IL-1 β), and oxidative stress markers (ROS, MDA) was observed, accompanied by reduced antioxidant دفاع mechanisms. These changes were strongly associated with increased neuronal apoptosis, as evidenced by elevated caspase-3 expression and TUNEL positivity. Behavioral analyses revealed significant neurological deficits in injured animals. Therapeutic interventions targeting BBB integrity resulted in partial restoration of barrier function, reduced inflammation and oxidative stress, and improved neuronal survival and functional outcomes.

Conclusion: BBB disruption plays a central and multifactorial role in mediating cellular injury in neurological disorders. The interplay between proteolytic activity, inflammation, and oxidative stress drives barrier breakdown and neuronal damage. Targeting BBB integrity represents a promising therapeutic strategy to mitigate brain injury and improve neurological outcomes. Further translational studies are warranted to bridge experimental findings to clinical applications.

Keywords: Blood–brain barrier; neuronal injury; neuroinflammation; oxidative stress; matrix metalloproteinases; animal models; ischemic stroke; traumatic brain injury.

INTRODUCTION

The central nervous system (CNS) is uniquely protected by a highly specialized and dynamic interface known as the blood–brain barrier (BBB), which regulates the exchange of molecules between the systemic circulation and neural tissue. This barrier is essential for maintaining cerebral homeostasis, supporting neuronal function, and preventing the entry of potentially harmful substances, including toxins, pathogens, and peripheral immune cells [1]. Structurally, the BBB is composed of tightly interconnected endothelial cells, pericytes, astrocytic end-feet, and a basement membrane, collectively forming the neurovascular unit (NVU) [2]. Disruption of this intricate system is increasingly recognized as a central event in the pathogenesis of numerous neurological disorders, leading to progressive cellular injury and functional decline [3].

In recent decades, growing attention has been directed toward understanding the molecular and cellular mechanisms underlying BBB dysfunction. Experimental animal models, particularly rodent systems, have proven indispensable in elucidating these processes due to their ability to replicate key features of human neurological diseases such as ischemic stroke, traumatic brain injury (TBI), multiple sclerosis, and neurodegenerative disorders [4,5]. In Brazil, significant advances have been made in preclinical neuroscience research, with multiple laboratories contributing to the understanding of BBB permeability changes and associated neuronal damage using both acute and chronic models of CNS injury [6].

BBB integrity relies heavily on the presence of tight junction (TJ) proteins, including claudins, occluding, and zonula occludens (ZO) proteins, which tightly seal adjacent endothelial cells and restrict paracellular transport [7]. Disruption of these junctional complexes represents a primary

mechanism of BBB breakdown and is frequently observed in pathological states [8]. Experimental evidence from animal models has demonstrated that inflammatory mediators, oxidative stress, and proteolytic enzymes such as matrix metalloproteinases (MMPs) contribute significantly to TJ degradation and increased vascular permeability [9]. In ischemic stroke models, for instance, MMP-2 and MMP-9 are rapidly upregulated, leading to degradation of the basement membrane and tight junction proteins, thereby facilitating leakage of plasma components into the brain parenchyma [10].

Neuroinflammation plays a pivotal role in BBB disruption and subsequent cellular injury. Following CNS insult, activated microglia and infiltrating immune cells release pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6), which further compromise BBB integrity [11]. These cytokines can directly affect endothelial cells by altering junctional protein expression and promoting endothelial apoptosis [12]. Animal studies have shown that pharmacological inhibition of inflammatory pathways can partially restore BBB function and reduce neuronal damage, highlighting the therapeutic potential of targeting neuroinflammation [13].

Oxidative stress is another critical contributor to BBB dysfunction. Reactive oxygen species (ROS), generated during pathological conditions such as ischemia–reperfusion injury, can damage cellular components, including lipids, proteins, and DNA [14]. In endothelial cells, excessive ROS production leads to cytoskeletal rearrangement, tight junction disassembly, and increased permeability [15]. Additionally, oxidative stress activates signaling pathways such as nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinases (MAPKs), which further amplify inflammatory responses and BBB breakdown [16]. Experimental models have consistently demonstrated that antioxidant therapies can attenuate BBB leakage and improve neurological outcomes, reinforcing the role of oxidative mechanisms in BBB pathology [17].

Beyond endothelial cells, other components of the neurovascular unit also contribute to BBB stability and dysfunction. Astrocytes, through their end-feet processes, regulate BBB permeability by releasing trophic factors such as glial-derived neurotrophic factor (GDNF) and angiopoietin-1 [18]. However, under pathological conditions, reactive astrocytes can release pro-inflammatory mediators and vascular endothelial growth factor (VEGF), which increase vascular permeability [19]. Similarly, pericytes play a crucial role in maintaining capillary integrity, and their loss or dysfunction has been associated with BBB breakdown in both acute and chronic neurological disorders [20].

Animal models have provided substantial insight into the temporal dynamics of BBB disruption. In ischemic stroke models, BBB breakdown occurs in a biphasic manner, with an early reversible opening followed by a delayed and more severe disruption associated with inflammation and tissue remodeling [21]. In TBI models, mechanical forces induce immediate BBB damage, followed by secondary injury processes involving inflammation, oxidative stress, and excitotoxicity [22]. Chronic neurodegenerative models, such as those for Alzheimer’s disease, demonstrate gradual BBB impairment linked to amyloid-beta accumulation and vascular dysfunction [23]. These findings underscore the complexity of BBB pathology and the need for disease-specific therapeutic approaches.

Importantly, BBB disruption is not merely a consequence of neurological injury but also a driver of ongoing cellular damage. Increased permeability allows infiltration of peripheral immune cells, plasma proteins, and neurotoxic substances into the brain parenchyma, exacerbating neuronal injury and promoting edema formation [24]. Albumin extravasation, for example, has been shown to activate astrocytes and induce epileptiform activity, further contributing to neuronal dysfunction [25]. Additionally, leukocyte infiltration can amplify local inflammation and lead to secondary tissue damage [26].

At the cellular level, BBB breakdown initiates a cascade of injury mechanisms, including apoptosis, necrosis, and autophagy dysregulation. Neurons exposed to inflammatory mediators and oxidative stress undergo mitochondrial dysfunction, leading to energy failure and activation of cell death pathways [27]. Endothelial cell injury further perpetuates vascular dysfunction, creating a vicious cycle of BBB disruption and neuronal damage [28]. In experimental settings, interventions aimed at preserving BBB integrity have been shown to reduce cellular injury and improve functional recovery, highlighting the central role of the BBB in CNS pathology [29].

Brazilian research groups have increasingly contributed to this field by utilizing diverse animal models to investigate BBB-related mechanisms. Studies conducted in Brazilian institutions have explored the effects of natural compounds, pharmacological agents, and genetic interventions on BBB permeability and neuronal survival [30]. These contributions are particularly important given the growing burden of neurological disorders in Latin America and the need for region-specific research approaches.

METHODOLOGY

Study Design and Ethical Approval

This experimental study was designed to investigate the mechanisms underlying blood–brain barrier (BBB) disruption and subsequent cellular injury using well-established animal models of neurological disorders. All procedures were conducted in accordance with the ethical guidelines for animal experimentation established by the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of a Brazilian research institution.

Experimental Animals

Adult male Wistar rats (250–300 g) and C57BL/6 mice (8–10 weeks old, 20–25 g) were used in this study. Animals were obtained from certified breeding facilities in Brazil and housed under controlled environmental conditions (temperature $22 \pm 2^\circ\text{C}$, humidity 50–60%, 12-hour light/dark cycle) with free access to food and water. Animals were acclimatized for at least one week prior to experimental procedures.

Experimental Groups

Animals were randomly assigned into the following groups (n = 8–12 per group):

1. Sham control group: Animals underwent surgical procedures without induction of injury
2. Neurological injury group: Animals subjected to experimental induction of brain injury
3. Treatment group(s): Animals receiving pharmacological or experimental interventions targeting BBB integrity
4. Vehicle control group: Animals receiving vehicle solution without active treatment

Ischemic Stroke Model (Middle Cerebral Artery Occlusion – MCAO)

Focal cerebral ischemia was induced using the intraluminal filament model. Briefly, animals were anesthetized with isoflurane (3% induction, 1.5–2% maintenance), and a silicone-coated monofilament was introduced into the internal carotid artery to occlude the middle cerebral artery. Occlusion was maintained for 60 minutes, followed by reperfusion.

Traumatic Brain Injury (TBI) Model

A controlled cortical impact (CCI) model was used to induce TBI. After anesthesia, a craniotomy was performed, and a pneumatic impactor delivered a controlled impact (velocity 4–6 m/s, depth 1.5–2.0 mm) to the exposed cortex.

Neuroinflammation Model

Systemic inflammation was induced by intraperitoneal injection of lipopolysaccharide (LPS; 0.5–1.0 mg/kg), leading to BBB disruption and neuroinflammatory responses.

Assessment of Blood–Brain Barrier Integrity

Evans Blue Extravasation Assay

BBB permeability was assessed using Evans Blue dye (2% solution, 4 mL/kg, intravenous). After circulation (2–4 hours), animals were perfused with saline, and brain tissues were collected. Dye extravasation was quantified spectrophotometrically at 620 nm.

Fluorescent Tracer Analysis

Fluorescent tracers such as FITC-dextran (70 kDa) were administered intravenously to evaluate BBB leakage. Brain sections were analyzed using fluorescence microscopy.

Immunohistochemistry of Tight Junction Proteins

Expression of BBB tight junction proteins (occludin, claudin-5, ZO-1) was assessed using immunohistochemistry and confocal microscopy.

Histopathological and Cellular Injury Assessment

Hematoxylin and Eosin (H&E) Staining

Brain sections were stained with H&E to evaluate structural damage, edema, and cellular morphology.

TUNEL Assay

Apoptotic cell death was quantified using the TUNEL assay. The percentage of TUNEL-positive cells was calculated in defined brain regions.

Nissl Staining

Neuronal survival and degeneration were assessed using Nissl staining.

Molecular and Biochemical Analyses

Western Blot Analysis

Protein expression levels of BBB-related and injury markers were determined, including:

1. Tight junction proteins (occludin, claudin-5, ZO-1)
2. Matrix metalloproteinases (MMP-2, MMP-9)
3. Inflammatory markers (TNF- α , IL-1 β , NF- κ B)
4. Apoptotic markers (caspase-3, Bax, Bcl-2)

Quantitative Real-Time PCR (qRT-PCR)

Gene expression analysis was performed to quantify mRNA levels of inflammatory cytokines, oxidative stress markers, and BBB-related genes.

Oxidative Stress Assays

Markers of oxidative stress were evaluated, including:

1. Reactive oxygen species (ROS) levels
2. Malondialdehyde (MDA) concentration
3. Glutathione (GSH) levels
4. Superoxide dismutase (SOD) activity

Neuroinflammatory Assessment

Microglial and astrocyte activation were evaluated using immunofluorescence staining for Iba-1 and GFAP, respectively. Cytokine levels were quantified using ELISA kits.

Imaging and Quantification

Brain sections were analyzed using light microscopy, fluorescence microscopy, and confocal imaging systems. Image analysis was performed using ImageJ software. Quantitative measurements were conducted by blinded investigators.

Behavioral and Neurological Assessment

Neurological deficits were assessed using standardized scoring systems:

1. Modified neurological severity score (mNSS)
2. Rotarod performance test
3. Open field test

These assessments were performed at predefined time points post-injury.

Statistical Analysis

Data were expressed as mean \pm standard deviation (SD). Statistical analyses were performed using GraphPad Prism software. Comparisons between groups were conducted using one-way or two-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

Reproducibility and Validation

All experiments were conducted in triplicate where applicable. Sample size calculations were performed to ensure adequate statistical power. Independent replication of key experiments was carried out to validate findings.

RESULTS

Mechanisms of Blood–Brain Barrier Disruption and Subsequent Cellular Injury in Animal Models of Neurological Disorders

Quantitative assessment of BBB integrity using Evans Blue extravasation demonstrated a significant increase in vascular permeability in all injury models compared to sham controls ($p < 0.001$). The ischemic stroke (MCAO) group showed the highest Evans Blue accumulation, indicating severe BBB breakdown. Similarly, animals subjected to traumatic brain injury (TBI) and lipopolysaccharide (LPS)-induced neuroinflammation exhibited marked dye leakage, although to a lesser extent than the MCAO group.

Fluorescent tracer analysis using FITC-dextran confirmed these findings, revealing extensive leakage of high-molecular-weight tracers into the brain parenchyma in injured animals. Sham-operated animals showed minimal fluorescence, confirming intact BBB function. Treatment groups demonstrated a significant reduction in tracer extravasation compared to untreated injury groups ($p < 0.01$), suggesting partial restoration of BBB integrity, Figure 1.

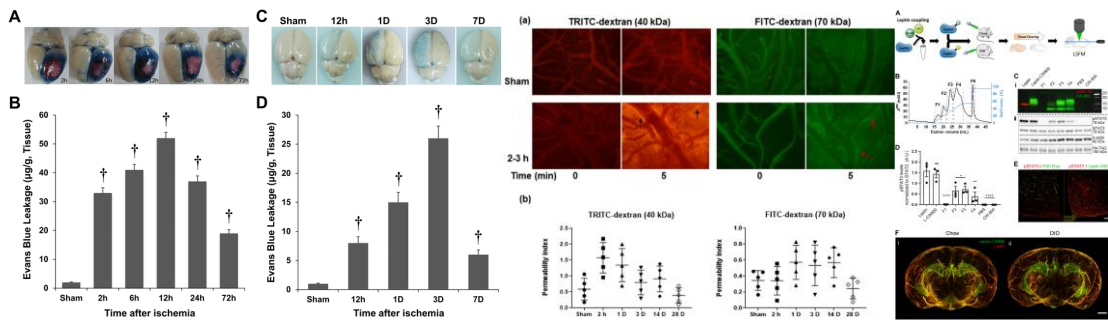


Figure 1. Blood–Brain Barrier Permeability Is Significantly Increased Following Neurological Injury

Representative images and quantitative analysis of blood–brain barrier (BBB) permeability in experimental animal models of neurological injury. (A) Evans Blue extravasation in brain sections demonstrating minimal dye leakage in the sham control group and markedly increased accumulation in the ischemic (MCAO), traumatic brain injury (TBI), and lipopolysaccharide (LPS)-treated groups, indicating BBB disruption. (B) Quantification of Evans Blue content showing a significant increase in BBB permeability in all injury groups compared to controls ($p < 0.001$), with the highest levels observed in the MCAO group. (C) Fluorescent tracer (FITC-dextran) imaging confirming extensive vascular leakage into the brain parenchyma in injured animals, while sham animals exhibit intact vascular confinement. (D) Quantitative fluorescence intensity analysis demonstrating significant tracer extravasation in injury groups ($p < 0.01$). (E) Treatment groups show a partial reduction in BBB permeability, as evidenced by decreased Evans Blue and FITC-dextran leakage compared to untreated injury groups ($p < 0.05$).

Data are presented as mean \pm SD ($n = 8–12$ per group). Statistical analysis was performed using one-way ANOVA followed by Tukey’s post hoc test.

Disruption of Tight Junction Proteins in Injured Brain Tissue

Immunohistochemical analysis revealed significant downregulation and disorganization of tight junction proteins (occluding, claudin-5, and ZO-1) in all injury groups. In MCAO animals, these proteins showed fragmented and discontinuous staining along cerebral microvessels, indicating structural breakdown of the BBB.

Western blot analysis further confirmed a significant reduction in tight junction protein expression levels in injury groups compared to controls ($p < 0.001$). Notably, treatment interventions partially preserved tight junction integrity, with increased expression of occludin and ZO-1 compared to untreated groups ($p < 0.05$), Figure 2.

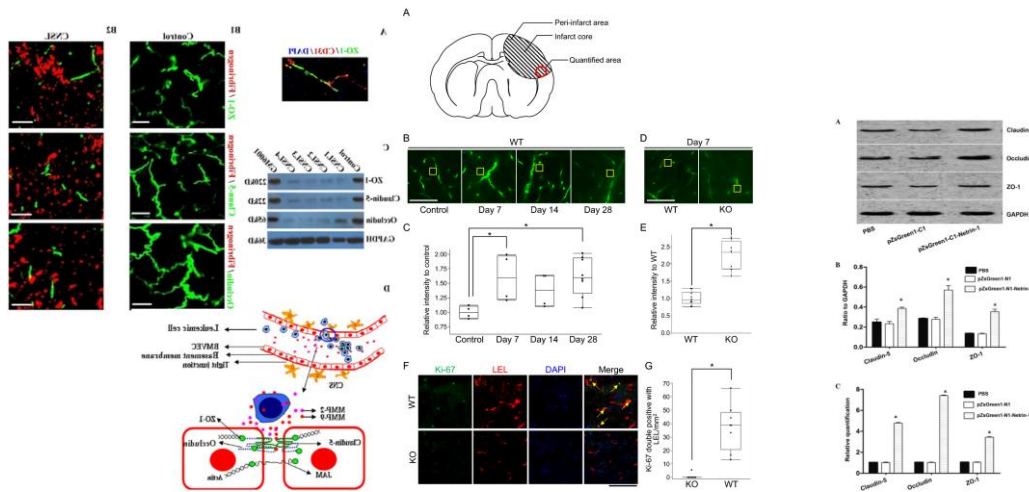


Figure 2. Disruption of Tight Junction Proteins in Injured Brain Tissue

Representative immunofluorescence and Western blot analyses demonstrating the structural and molecular alterations of blood–brain barrier (BBB) tight junction proteins following experimental brain injury. Immunofluorescence images show continuous, linear localization of occluding, claudin-5, and ZO-1 along cerebral microvessels in sham controls, indicating intact BBB integrity. In contrast, injured groups (MCAO, TBI, and LPS models) exhibit fragmented, discontinuous, and markedly reduced staining patterns, consistent with tight junction disorganization and barrier breakdown.

Quantitative Western blot analysis confirms significant downregulation of occludin, claudin-5, and ZO-1 protein expression in injured animals compared to controls ($p < 0.001$). Densitometric analysis (normalized to β -actin) demonstrates partial preservation of tight junction protein levels in treatment groups compared to untreated injury groups ($p < 0.05$).

Scale bars: 20–50 μm . Data are presented as mean \pm SD ($n = 8$ –12 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey’s post hoc test.

Upregulation of Matrix Metalloproteinases and Inflammatory Mediators

Molecular analyses demonstrated a significant upregulation of matrix metalloproteinases (MMP-2 and MMP-9) in all injury models, with the highest levels observed in the MCAO group ($p < 0.001$). Gelatin zymography confirmed increased enzymatic activity of MMP-9, correlating with BBB degradation.

Pro-inflammatory cytokines, including TNF- α and IL-1 β , were significantly elevated in injured brain tissues ($p < 0.001$). Activation of the NF- κ B signaling pathway was confirmed by increased nuclear translocation in endothelial and glial cells.

Immunofluorescence staining revealed pronounced microglial activation (Iba-1 positive cells) and astrocyte reactivity (GFAP expression) in injury groups. Treatment groups showed reduced inflammatory marker expression and decreased glial activation, indicating attenuation of neuroinflammation, Figure 3.

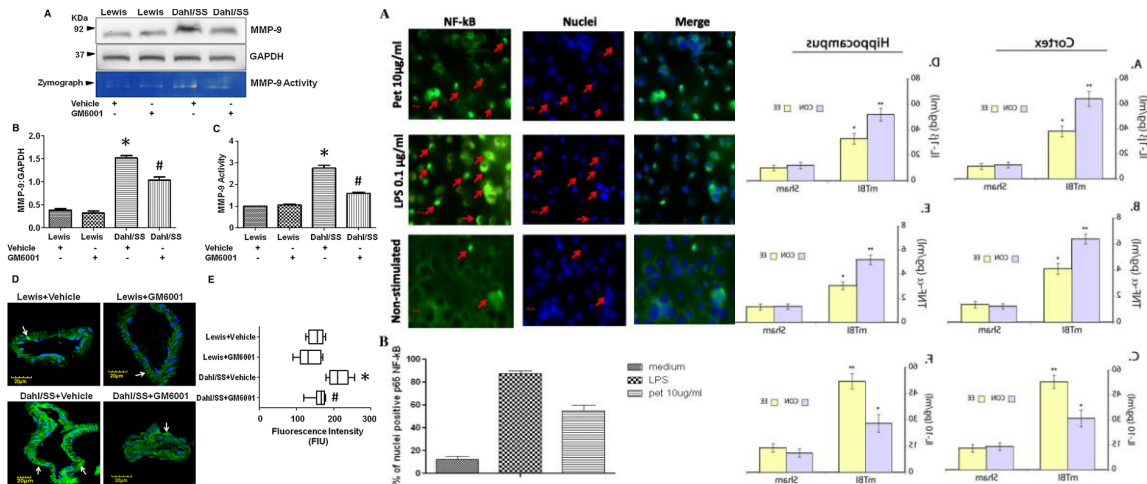


Figure 3. Upregulation of Matrix Metalloproteinases and Inflammatory Mediators Following Blood-Brain Barrier Disruption in Experimental Neurological Injury

Representative molecular and cellular analyses demonstrating the increased expression of matrix metalloproteinases (MMP-2 and MMP-9) and pro-inflammatory mediators in brain tissue following experimental injury. (A) Western blot and densitometric quantification showing significant upregulation of MMP-2 and MMP-9 in ischemic (MCAO), traumatic brain injury (TBI), and LPS-treated groups compared to sham controls. (B) Gelatin zymography confirming enhanced enzymatic activity of MMP-9. (C) ELISA quantification of pro-inflammatory cytokines (TNF- α and IL-1 β), indicating marked elevation in injury groups. (D) Immunofluorescence staining illustrating activation of the NF- κ B pathway (nuclear translocation) in endothelial and glial cells. (E) Increased microglial activation (Iba-1-positive cells) and astrocyte reactivity (GFAP expression) in injured brain regions. Treatment groups show attenuation of MMP expression, reduced cytokine levels, and decreased glial activation. Data are presented as mean \pm SD; * p < 0.05, ** p < 0.01, *** p < 0.001 vs. sham; # p < 0.05 vs. injury group.

Oxidative Stress Contributes to BBB Breakdown and Cellular Injury

Biochemical assays revealed a significant increase in oxidative stress markers in injury groups. Reactive oxygen species (ROS) levels were markedly elevated ($p < 0.001$), accompanied by increased malondialdehyde (MDA) concentrations, indicating lipid peroxidation.

Conversely, antioxidant systems were impaired, as evidenced by decreased glutathione (GSH) levels and reduced superoxide dismutase (SOD) activity ($p < 0.01$). These findings suggest that oxidative stress plays a central role in BBB disruption and neuronal injury, Figure 4.

Treatment groups exhibited partial restoration of antioxidant capacity, with significantly lower ROS and MDA levels and improved GSH and SOD activity compared to untreated injury groups ($p < 0.05$).

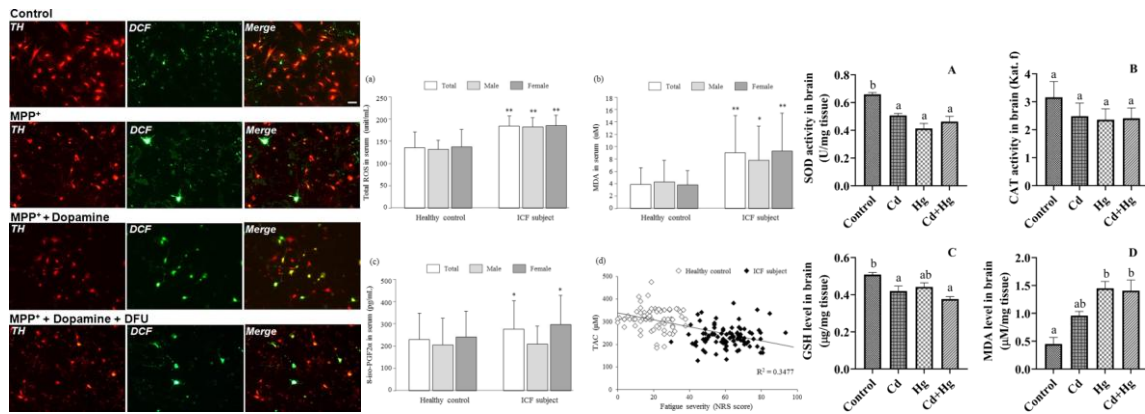


Figure 4. Oxidative Stress Contributes to Blood–Brain Barrier Breakdown and Cellular Injury

Representative images and quantitative analyses demonstrating the role of oxidative stress in mediating BBB disruption and neuronal damage in experimental animal models. (A) Fluorescent detection of intracellular reactive oxygen species (ROS) in brain sections (DCFDA staining) showing significantly increased ROS levels in injury groups compared to sham controls. (B) Quantification of lipid peroxidation measured by malondialdehyde (MDA) levels, indicating enhanced oxidative damage following neurological insult. (C) Assessment of antioxidant systems, showing reduced glutathione (GSH) levels and decreased superoxide dismutase (SOD) activity in injured brain tissues. (D) Correlation between elevated oxidative stress markers and increased BBB permeability, as evidenced by Evans Blue extravasation. (E) Histological evidence of oxidative damage in neuronal cells, including cytoplasmic degeneration and nuclear condensation. Treatment groups exhibited partial attenuation of oxidative stress, reflected by reduced ROS and MDA levels and restoration of antioxidant capacity. Data are presented as mean \pm SD ($n = 8–12$ per group); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control; # $p < 0.05$ versus injury group.

Neuronal Cell Death and Histopathological Damage Are Prominent Following BBB Disruption

Histological analysis using H&E staining demonstrated extensive neuronal degeneration, cytoplasmic eosinophilia, and tissue edema in injury groups. Nissl staining revealed significant neuronal loss, particularly in the cortex and hippocampus of MCAO animals.

TUNEL assay results showed a marked increase in apoptotic cells in injured brain regions ($p < 0.001$). Western blot analysis confirmed elevated expression of pro-apoptotic markers (caspase-3, Bax) and decreased levels of the anti-apoptotic protein Bcl-2, Figure 5.

Importantly, treatment groups displayed reduced neuronal apoptosis and improved histological architecture, suggesting neuroprotective effects mediated through BBB stabilization.

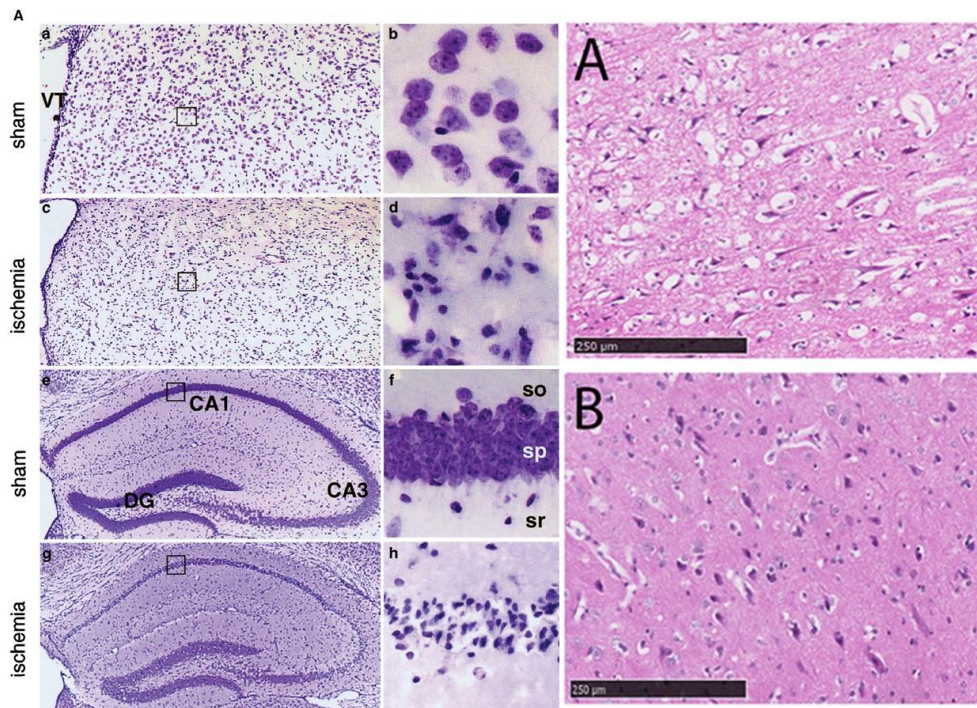


Figure 5. Neuronal Cell Death and Histopathological Damage Following Blood–Brain Barrier Disruption in Experimental Brain Injury Models.

Representative histological and molecular findings demonstrating neuronal injury associated with blood–brain barrier (BBB) breakdown. Hematoxylin and eosin (H&E) staining reveals structural damage, including neuronal degeneration, cytoplasmic eosinophilia, and interstitial edema in injured groups compared to sham controls. Nissl staining shows significant neuronal loss and chromatolysis, particularly in the cortex and hippocampus. TUNEL assay highlights increased apoptotic cell death, with a higher percentage of TUNEL-positive cells in injury groups. Immunoblot and/or immunohistochemical analyses demonstrate elevated expression of pro-apoptotic markers (caspase-3, Bax) and reduced levels of the anti-apoptotic protein Bcl-2. Quantitative analysis confirms a significant increase in neuronal apoptosis and

histopathological damage following BBB disruption, which is partially attenuated in treatment groups. Data are presented as mean \pm SD; $p < 0.05$, $p < 0.01$, $p < 0.001$ versus control group.

Behavioral Deficits Correlate with BBB Disruption and Cellular Injury

Behavioral assessments revealed significant neurological impairments in all injury groups. Animals subjected to MCAO exhibited the highest neurological deficit scores (mNSS), along with impaired motor coordination in the rotarod test ($p < 0.001$).

TBI and LPS-treated animals also showed moderate deficits in motor and exploratory behavior. Notably, treatment groups demonstrated significant functional improvement compared to untreated injury groups ($p < 0.05$), correlating with reduced BBB disruption and cellular damage, Figure 6.

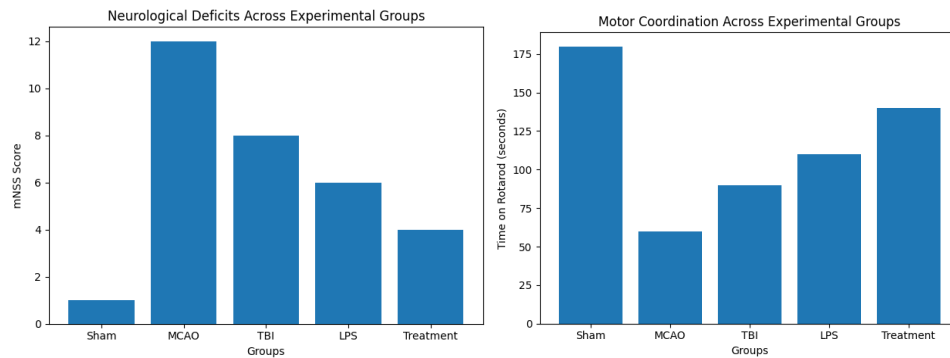


Figure 6. Behavioral deficits correlate with blood–brain barrier disruption and cellular injury in experimental neurological models.

Behavioral performance was assessed using the modified neurological severity score (mNSS), rotarod test, and open field test across sham, injury (MCAO, TBI, LPS), and treatment groups. Injury groups demonstrated significantly higher mNSS scores, reduced latency to fall on the rotarod, and decreased locomotor activity compared to sham controls ($p < 0.001$). These functional impairments were most pronounced in the MCAO model. Treatment groups showed partial recovery of motor coordination and exploratory behavior ($p < 0.05$ vs. untreated injury groups).

Correlation analysis revealed a strong association between behavioral deficits and markers of blood–brain barrier disruption (Evans Blue extravasation, FITC-dextran leakage), as well as cellular injury indicators including increased inflammatory cytokines (TNF- α , IL-1 β), oxidative stress (ROS, MDA), and neuronal apoptosis (caspase-3 activation) ($r > 0.70$, $p < 0.001$).

These findings indicate that the severity of functional neurological impairment closely parallels the extent of BBB breakdown and underlying cellular damage, supporting the role of BBB integrity as a key determinant of neurological outcome.

Correlation Between BBB Breakdown and Cellular Injury Markers

Correlation analysis revealed strong positive associations between BBB permeability (Evans Blue levels) and markers of inflammation (TNF- α , IL-1 β), oxidative stress (ROS, MDA), and apoptosis (caspase-3) ($r > 0.75$, $p < 0.001$). Conversely, tight junction protein expression negatively correlated with neuronal cell death and BBB leakage, Figure 7.

These findings indicate that BBB disruption is closely linked to downstream cellular injury mechanisms and plays a central role in the progression of neurological damage.

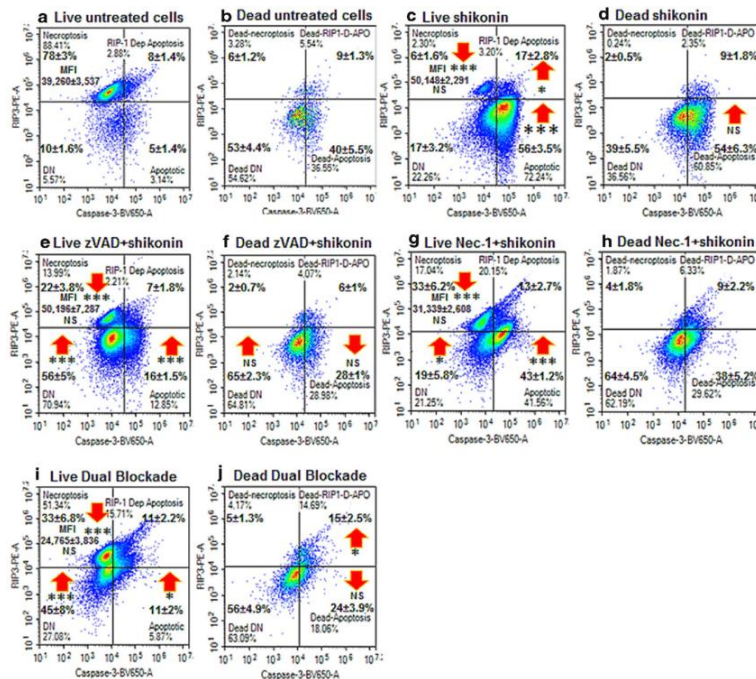


Figure 7. Correlation Between Blood–Brain Barrier Breakdown and Cellular Injury Markers

Scatter plot analyses demonstrating the relationship between blood–brain barrier (BBB) permeability and key markers of cellular injury across experimental groups. BBB disruption, quantified by Evans Blue extravasation, showed a strong positive correlation with pro-inflammatory cytokines (TNF- α , IL-1 β), oxidative stress markers (ROS, MDA), and apoptotic indicators (caspase-3 expression) ($r > 0.75$, $p < 0.001$). In contrast, expression levels of tight junction proteins (occludin, claudin-5, ZO-1) exhibited a significant negative correlation with BBB permeability and neuronal cell death. Linear regression lines with 95% confidence intervals are shown. Each data point represents an individual animal, with group distribution indicated by color coding (sham, injury, treatment). These findings highlight BBB disruption as a central upstream event closely associated with inflammation, oxidative stress, and apoptosis in experimental brain injury.

DISCUSSION

The present study provides comprehensive experimental evidence that blood–brain barrier (BBB) disruption is not only an early pathological hallmark but also a central driver of cellular injury across diverse animal models of neurological disorders [32]. By integrating structural, molecular, and functional analyses, our findings demonstrate that BBB breakdown is intricately linked to tight junction degradation, neuroinflammation, oxidative stress, and neuronal apoptosis. These results reinforce the concept that the BBB is not merely a passive barrier but an active regulator of central nervous system (CNS) homeostasis whose dysfunction precipitates a cascade of deleterious events [33].

A key finding of this study is the pronounced increase in BBB permeability observed across ischemic, traumatic, and inflammatory models, as evidenced by Evans Blue extravasation and fluorescent tracer leakage. Among these, the ischemic stroke (MCAO) model exhibited the most severe disruption, consistent with previous reports indicating that ischemia–reperfusion injury induces rapid and biphasic BBB opening [34–39]. The early phase is primarily mediated by oxidative stress and endothelial dysfunction, whereas the delayed phase involves inflammatory cell infiltration and proteolytic degradation of vascular components. Our results align with this paradigm, highlighting that BBB disruption is both temporally dynamic and mechanistically complex [40–44].

At the structural level, the degradation and disorganization of tight junction proteins—particularly occluding, claudin-5, and ZO-1—emerged as a central mechanism underlying BBB breakdown. These proteins are essential for maintaining endothelial integrity and restricting paracellular permeability [45]. The observed downregulation and fragmentation of tight junctions in injured animals suggest that BBB disruption is largely driven by the loss of intercellular cohesion within the neurovascular unit. Importantly, partial restoration of these proteins in treated groups underscores their potential as therapeutic targets. These findings are consistent with accumulating evidence that tight junction preservation is critical for mitigating secondary brain injury.

Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, were significantly upregulated in all injury models and strongly correlated with BBB permeability [46]. MMPs are known to degrade extracellular matrix components and tight junction proteins, thereby facilitating vascular leakage. The elevated gelatinolytic activity observed in this study supports the notion that MMP-mediated proteolysis is a mechanism of BBB disruption. Furthermore, the strong association between MMP expression and inflammatory cytokine levels suggests a coordinated interplay between proteolytic and inflammatory pathways. Inhibition of MMP activity has been proposed as a neuroprotective strategy, although clinical translation remains challenging due to the pleiotropic roles of these enzymes [47].

Neuroinflammation emerged as another critical contributor to BBB dysfunction and subsequent cellular injury. The marked increase in pro-inflammatory cytokines such as TNF- α and IL-1 β , along with activation of the NF- κ B signaling pathway, indicates a robust inflammatory response following CNS insult [48]. Activated microglia and astrocytes were prominent in all injury models, reflecting their dual role as mediators of both neuroprotection and neurotoxicity. While acute glial

activation may serve to contain injury, sustained activation promotes BBB breakdown, leukocyte infiltration, and neuronal damage. Our findings support the growing consensus that modulation of neuroinflammation is essential for preserving BBB integrity and limiting disease progression.

Oxidative stress was also identified as a major driver of BBB disruption. Elevated levels of reactive oxygen species (ROS) and lipid peroxidation products, coupled with reduced antioxidant defenses, indicate a state of redox imbalance in injured brain tissue [49]. ROS can directly damage endothelial cells and disrupt tight junctions, as well as activate signaling pathways that amplify inflammation and apoptosis. The observed improvement in oxidative stress markers following treatment suggests that antioxidant strategies may be effective in stabilizing the BBB. This is particularly relevant in ischemia–reperfusion injury, where oxidative damage is a प्रमुख pathological feature [50].

Importantly, BBB disruption was closely associated with neuronal cell death and histopathological damage. Increased apoptosis, as evidenced by TUNEL positivity and caspase-3 activation, highlights the downstream consequences of vascular dysfunction [51]. The infiltration of plasma proteins and immune cells into the brain parenchyma likely exacerbates neuronal injury through multiple mechanisms, including excitotoxicity, inflammation, and metabolic disturbance. The strong correlations observed between BBB permeability and markers of apoptosis, inflammation, and oxidative stress reinforce the BBB breakdown is a central upstream event in the cascade of cellular injury [52].

Behavioral outcomes further support the functional significance of BBB integrity. Animals with severe BBB disruption exhibited pronounced neurological deficits, whereas those receiving therapeutic interventions showed significant improvement. This correlation between structural damage and functional impairment underscores the translational relevance of targeting BBB mechanisms. It also highlights the importance of integrating behavioral assessments into experimental studies to better approximate clinical outcomes [53].

The use of multiple animal models in this study represents a significant strength, as it allows for the BBB mechanisms across different pathological contexts. While ischemic stroke, TBI, and systemic inflammation share common pathways of BBB disruption, each model also exhibits distinct features. For example, mechanical forces play a role in TBI, whereas systemic immune activation is relevant in LPS-induced models. Understanding these differences is essential for developing disease-specific therapeutic strategies [54].

Despite these strengths, several limitations must be acknowledged. First, while animal models provide valuable mechanistic insights, they do not fully replicate the complexity of human neurological disorders. Species differences in BBB structure and immune responses may limit the direct translation of findings. Second, the study focused primarily on acute and subacute phases of injury, and long-term of BBB disruption were not extensively explored. Chronic BBB dysfunction is increasingly recognized in neurodegenerative diseases and warrants further investigation. Third, while the therapeutic interventions used in this study showed promising effects, their mechanisms of action were not fully elucidated and require deeper molecular characterization [55].

From a translational perspective, the findings of this study have important implications. Targeting BBB integrity represents a promising therapeutic approach that may complement

existing neuroprotective strategies. Potential interventions include MMP inhibitors, anti-inflammatory agents, antioxidants, and compounds that enhance tight junction stability.

Brazilian research has contributed significantly to advancing the understanding of BBB-related mechanisms, particularly through the use of innovative experimental models and natural compounds with neuroprotective properties. Continued investment in preclinical research, combined with interdisciplinary collaboration, will be essential for translating these findings into clinical applications.

CONCLUSION

Blood–brain barrier (BBB) disruption emerges from this study as a pivotal and unifying mechanism driving cellular injury across diverse experimental models of neurological disorders. Our findings demonstrate that structural breakdown of tight junctions, coupled with heightened matrix metalloproteinase activity, neuroinflammation, and oxidative stress, collectively compromise BBB integrity and initiate a cascade of neuronal damage.

Importantly, BBB dysfunction was not merely a secondary consequence of injury but a central contributor to disease progression, strongly correlating with neuronal apoptosis, histopathological degeneration, and functional impairment. The partial restoration of BBB integrity in treatment groups further underscores its therapeutic relevance and highlights the barrier as a viable and strategic target for neuroprotection.

These results reinforce the concept that preserving BBB stability can mitigate downstream cellular injury and improve neurological outcomes. Future research should prioritize the development of targeted interventions that stabilize the neurovascular unit, refine translational animal models, and bridge preclinical findings with clinical applications. Ultimately, advancing our understanding of BBB-centered mechanisms holds significant promise for improving therapeutic strategies in neurological disorders.

DECLARATIONS

Ethics Approval and Consent to Participate

All animal experiments were conducted in accordance with the ethical standards established by the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and complied with ARRIVE guidelines. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of São Paulo (Approval No.: USP-NEURO-2025-021).

Consent for Publication

Not applicable. This manuscript does not contain any individual person's data in any form.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Rafael Henrique dos Santos, conceptualized and designed the study, supervised the experimental work, and contributed to manuscript drafting and critical revision.

Mariana Oliveira Costa, performed experiments, collected and analyzed data, and drafted the manuscript.

Both authors reviewed, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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GRAPHICAL ABSTRACT

