ABSTRACT

Traumatic cerebral vascular injury (TCVI) is a frequent, perhaps universal, feature after traumatic brain injury (TBI) and may be responsible for some TBI-related chronic disabilities. Because there are multiple pharmacologic and non-pharmacologic therapies that promote vascular health, TCVI is an attractive target for therapeutic intervention after TBI. The cerebral microvasculature (CMV) is a component of the neurovascular unit (NVU) coupling neuronal metabolism with local cerebral blood flow. The NVU participates in the pathogenesis of TBI, either directly from physical trauma or as part of the cascade of secondary injury that occurs after TBI. Pathologically, there is extensive microvascular injury in humans and experimental animals, identified with either conventional light microscopy or ultrastructural examination. It is seen in acute and chronic TBI and even described in chronic traumatic encephalopathy (CTE). Non-invasive, physiologic measures of cerebral microvascular function show dysfunction after TBI in humans and experimental animal models of TBI. These include imaging sequences Arterial Spin Labeling (ASL), Transcranial Doppler, Near InfraRed Spectroscopy (NIRS), etc. Understanding the pathophysiology of TCVI, a relatively under-studied component of TBI, has promise for developing novel TBI therapies.

Introduction

TBI places an enormous burden on patients and society, and has led to extensive predilection research and numerous, unsuccessful clinical trials. While the complex molecular and cellular mechanisms responsible for TBI-associated deficits are incompletely understood, substantial data suggest that traumatic cerebral vascular injury (TCVI), at least partially, underlies a significant fraction of TBI-related disability. Because the cerebral vasculature is highly plastic, TCVI is an attractive target for therapies. There are well established pharmacologic and non-pharmacologic approaches that promote vascular health, such as PDE-5 inhibitors, HMG-CoA reductase inhibitors, HDL mimetics, and PPAR-γ agonists, among others. This review will focus on TCVI preclinical and clinical data.

The neurovascular unit

The micro-network regulating cerebral blood flow, vascular permeability and angiogenesis has been coined the neurovascular unit (NVU). It actively participates in the pathogenesis of many brain disorders, including common conditions such as hypertension, diabetes and neurodegenerative disorders (e.g. Alzheimer’s disease). The NVU has been an intense focus of research in multiple acute and chronic neurologic disorders.

Pathophysiology of NVU injury after TBI

TCVI can result from both primary and secondary injury (e.g. blood brain barrier disruption, increased intracellular...
calcium, mitochondrial dysfunction, neuroinflammation)\textsuperscript{7}. After TBI, the changes seen in the BBB have a biphasic mode of action–immediate changes caused by direct damage to endothelial cells followed by changes from secondary injuries in other elements of the NVU (neurons, astrocytes, pericytes, microglia and the extracellular matrix)\textsuperscript{3}. Diminished CBF and focal tissue hypoxia is a common precipitant of NVU pathophysiology and is mediated through multiple pathophysiologic cascades (e.g. BBB disruption, edema, focal ischemia)\textsuperscript{3,9,10}. When injured, the NVU rapidly increases blood flow and oxygen supply and induces factors that promote angiogenesis\textsuperscript{8,11}. However, the NVU also attempts self-repair through mechanisms with potential deleterious consequences that enhance secondary injury if homeostasis is not quickly restored\textsuperscript{8}. These changes often occur remote from the TBI impact and represent secondary NVU changes.

**Pathology of microvascular injury in acute TBI—primary injury**

**Preclinical studies of acute changes**

Microvascular injury is a near universal finding in experimental TBI, and has been reported in nearly all animal models, including impact acceleration\textsuperscript{12,13}, fluid percussion injury\textsuperscript{14}, and controlled cortical impact (CCI)\textsuperscript{15}. Early studies with fluid percussion injury showed pericontusion petechial hemorrhages around small venules, pyknotic neurons, and swollen astrocytes. Ultrastructural analysis revealed early vessel wall damage in areas with irreversible neuronal injury\textsuperscript{16}. Another fluid percussion injury study showed reduced microvascular density (57% loss) within cortical contusions\textsuperscript{17}. After CCI, acute migration of pericytes was observed from microvascular and induces factors that promote angiogenesis\textsuperscript{8,11}. However, the NVU also attempts self-repair through mechanisms with potential deleterious consequences that enhance secondary injury if homeostasis is not quickly restored\textsuperscript{8}. These changes often occur remote from the TBI impact and represent secondary NVU changes.

**Pathology of microvascular injury in chronic TBI—CMV repair**

Injured blood vessels respond to TBI through local repair (Table 1). The CMV affects this through increased

**Human neuropathological observations in acute/subacute TBI**

TCVI is a near universal feature of severe TBI\textsuperscript{13,22}. There are abundant pathological reports describing TCVI after fatal TBI\textsuperscript{23}, and, although less frequently, also in individuals who died from non-TBI related complications after mild TBI\textsuperscript{11,24}. Microscopic perivascular hemorrhages are seen even when macroscopic hemorrhage is absent. Microscopically, there are abundant intravascular microthrombi in the microvasculature. In samples from non-contused or contused sections after fatal TBI cases, intravascular microthrombi are seen in both, only varying in density, and correlated with focal areas of neuronal necrosis\textsuperscript{26}, suggesting a possible link between microthrombi and neuronal death\textsuperscript{16}. Rodríguez-Baeza and colleagues studied the CMV ultrastructurally in 10 TBI patients who died between 1 and 20 days after injury\textsuperscript{11}. CMV corrosion casts revealed 3 changes in the arterioles and capillaries of the middle and deep cortical vascular zones in TBI brains: 1) longitudinal folds; 2) sunken vascular surfaces with craters at endothelial junctions; 3) reduction of the vessel lumen (Figure 1)\textsuperscript{11}.

Recent electron microscopy studies after severe TBI describe the following capillaries and pericytes changes: thickening of the basement membrane 3 to 8 times normal; rarefaction, vacuolization and splitting of the capillary basement membrane; pericyte hypertrophy; pericyte rarefaction and necrosis; lipofuscin and lipid deposits in pericyte cytoplasm\textsuperscript{25,26}, indicating widespread microvascular injury after TBI\textsuperscript{11,25,26}. Injured blood vessels respond to TBI through local repair (Table 1). The CMV affects this through increased

**Figure 1:** Scanning electron micrographs showing microvascular changes in 2 arteriolar vessels from the frontal lobe after TBI. a: Arteriole with longitudinal folds (2') with transition from an undamaged, smooth vessel (1) to an injured, folded vessel (2) (arrowheads indicate the transition point). 1 = subpial zone; 2 = superficial zone; 3 = cortical zone. Scale bar = 86 μm. b: Arteriolar vessels (1) with longitudinal folds (2) and nuclear imprints of endothelial cells (3) at higher power. Scale bar = 23 μm. Ref: Rodríguez-Baeza A, Reina-de la Torre F, Poca A et al. Morphological features in human cortical brain microvessels after head injury: a three-dimensional and immunocytochemical study. Anat Rec A Discov Mol Cell Evol Biol 2003; 273:583–593.
protein synthesis along with cell hypertrophy and hyperplasia. These time-dependent responses are not seen until 3 hours to 7 weeks after TBI and can be used in forensic injury dating. In animal models, therapies that promote CMV repair and/or angiogenesis, such as statins or sildenafil, promote neurologic recovery.

**Pathology of cerebral microvascular injury in chronic traumatic encephalopathy (CTE)**

Prominent microvasculopathy is also described in CTE. Cortical vascular changes, such as thickened perforating arteries, absence of nuclei in vascular cells, and diffuse hyaline, were described in the initial CTE cases. Among CTE cases in the Corsellis collection diffuse perivascular hemosiderin collections in macrophages, neuroglia or extracellular space were described. In sports-related CTE cases, striking vascular changes such as perivascular microgliosis and astrocytosis, neurofibrillary tangles, and spindle-shaped neurites in the sulcal depths of cortical gyri, and hemosiderin-laden perivascular macrophages have been described. In a series of blast-associated CTE cases, McKeever similarly noted perivascular lymphocytic cuffing, and hemosiderin-laden macrophages within cerebral vessel walls and focal calcifications of penetrating small thalamic and deep white matter vessels similar to that seen in mouse models of TCVI.

**Non-invasive assessment of TCVI.**

There are two methods to assess CMV function: functional neuroimaging and cerebrovascular reactivity (CVR) measurements. Novel neuroimaging sequences have been coupled with dynamic procedures that increase physiologic demand to measure the CMV’s ability to respond and indirectly TCVI.

**Neuroimaging and CVR in experimental animals**

Neuroimaging studies consistently show reduced cerebral blood flow (CBF) acutely after experimental TBI by functional MRI and laser Doppler flowmetry. In fluid percussion injury, CBF measured via continuous ASL, showed reductions the first 2 weeks, corresponding with decreases in cortical small vessel density. CVR can be assessed in experimental models through cranial windows that allow direct visualization of the pial microvasculature. The anesthetized animals undergo a hypercapnia challenge with 3-5% carbon dioxide (CO2) while the pial microvasculature is assessed. One week after injury, there is a significant decrease in CVR compared to sham injured controls. Other studies report that TBI causes a loss of the normal vasodilatory response to potent vasodilators (acetylcholine, adenosine, and sodium nitroprusside).

**Neuroimaging studies in humans**

CBF has been extensively studied after TBI in humans, especially in the acute period, when CBF deficits are common. Bonne et al. used single photon emission computed tomography (SPECT) to measure regional CBF in symptomatic, chronic TBI patients and found areas of cerebral hypoperfusion. With SPECT, Lewine et al. described CBF abnormalities in 40% of 30 chronic symptomatic mild TBI patients and found SPECT to be significantly more sensitive than MRI. A recent meta-review concluded that SPECT outperformed CT and MRI in both acute and chronic TBI diagnosis.

Other advanced MRI techniques have been helpful in evaluating TCVI. ASL provides a direct measurement of arterial perfusion in absolute units of CBF. Kim et al. showed that chronic moderate or severe TBI patients have reduced global CBF in the resting state, as well as decreased regional perfusion in the thalamus, posterior cingulate cortex, and frontal cortex. Regional CBF can also be calculated by perfusion-weighted imaging (PWI). In 15 symptomatic sports-related concussion patients studied 6 months post-concussion, PWI showed reduced CBF in the thalami bilaterally and reduced cerebral blood volume in the left thalamus compared to controls. Susceptibility-weighted imaging (SWI) detects microbleeds better than gradient recalled echo MRI in traumatic axonal injury, and the total number and volume of microbleeds correlate with TBI-associated functional outcomes.

**Assessment of cerebrovascular reactivity in humans.**

Several methods exist to study CVR non-invasively in humans after hypercapnia, breath-holding, or acetazolamide, using Transcranial Doppler (TCD), functional MRI and near infra-red spectroscopy (NIRS). While TCD and NIRS offer the advantage of high temporal resolution, MRI offers superb spatial resolution. A prospective study of 299 moderate-to-severe TBI patients assessed cerebral vasospasm with TCD. Nearly half (45.2%) of the patients had vasospasm with the highest risk at day 3 after injury. CVR, measured by both TCD and NIRS, was decreased in 12 professional boxers 72 hours after a bout. Compared to controls the boxers also had chronically impaired CVR by both modalities and lower CVR measurements correlated with increased neurocognitive dysfunction and inversely correlated with TBI exposure. A meta-analysis reported reduced CVR via TCD in 42 athletes examined between 2 and 5 days after sports-related concussion.

NIRS, another noninvasive measure of CVR, is currently...
also being used to assess CMV function in TBI. NIRS allows CVR measurements during dynamic challenges that are independent of hemoglobin concentration, skull thickness and extracranial circulation. Using a NIRS-based CVR index in 40 acute TBI patients, total hemoglobin reactivity index measured by NIRS correlated with the intracranial pressure derived cerebrovascular pressure reactivity index. A study of 37 critically ill TBI patients showed a good correlation between hemoglobin reactivity index measured via NIRS and intracranial pressure.

**Hypercapnia-BOLD MRI reliably measures deficits in CVR in the chronic stage after TBI**

Direct measures of microvascular injury require assessment of CVR using manipulations that directly affect endothelial function. One method combines MRI with the Blood Oxygen Dependent (BOLD) signal in response to hypercapnia challenge. The hypercapnia challenge is administered during the MRI by the inhalation of room air alternating with room air + 5% CO₂. The CVR is the ratio of change in BOLD signal to the change in end-tidal CO₂ and with voxel-by-voxel measurements, a CVR map can be drawn.

Our group recently completed a study of CVR by MRI-BOLD with hypercapnia challenge in 27 chronic stage TBI patients (15 controls). There was significantly decreased CVR in the chronic TBI subjects (Figure 4). We adapted the hypercapnia challenge to NIRS testing and found similar CVR reductions in chronic TBI, and a strong correlation with the MRI-BOLD results (Figure 5). These results support the persistence of TCVI into the chronic stage and the reliable use of both NIRS and MRI. CVR may prove to be a useful predictive and pharmacodynamic biomarker in therapeutic trials of TCVI.

**CONCLUSIONS**

TCVI has been an under-recognized TBI phenotype despite a robust theoretical construct and a large body of empirical evidence. The studies reviewed here support the hypothesis that TCVI is near ubiquitous after TBI. Further, it plays a potentially important role in chronic post-concussive symptoms and even TBI-associated neurodegenerative disorders. Further neuropathological studies are required.

![Figure 2](image-url)
**Figure 3:** T1 MRI images (rows 1 & 3) with corresponding cerebrovascular reactivity (CVR) maps (rows 2 & 4) based on MRI-BOLD with 5% hypercapnia challenge in a healthy control (top 2 rows) compared to chronic TBI subject (bottom 2 rows). Note the relatively uniform CVR throughout the cortex in the control subject in the top two rows compared to the patchy, moth-eaten appearance to the CVR maps from the TBI subjects. As expected, in areas of visible encephalomalacia, the CVR was decreased. However, there were also many regions where the CVR was depressed but the structural MRI was normal. Ref: Courtesy of Franck Amyot, Ph.D., CNRM, USUHS.

**Figure 4:** Cerebrovascular reactivity (CVR) histogram via MRI-BOLD with 5% CO₂ hypercapnia challenge in moderate and severe traumatic brain injury patients (red) compared to age-matched healthy controls (blue). The CVR histogram in TBI patients is both shifted to the left and has much greater variance. The CVR histogram in healthy controls is remarkably stable with very little intersubject variability. Ref: Kenney K, Amyot F, Haber M, et al. Cerebral Vascular Injury in Traumatic Brain Injury. Exp Neurol. 2016;275 Pt 3:353-366. Courtesy of Franck Amyot, Ph.D., CNRM, USUHS.

**Figure 5:** Correlation of CVR measurements via MRI-BOLD (with 5% CO₂ hypercapnia challenge via the Douglas Bag method) and fNIRS (Near InfraRed Spectroscopy with 5% CO₂ hypercapnia challenge via the Douglas Bag method) in traumatic brain injury (TBI) and healthy controls. The complementary methods give similar results with a high degree of correlation (R = 0.71) with lower and more variable CVR measurements in TBI patients compared to healthy controls. Ref: Kenney K, Amyot F, Haber M, et al. Cerebral Vascular Injury in Traumatic Brain Injury. Exp Neurol. 2016;275 Pt 3:353-366. Courtesy of Franck Amyot, Ph.D., CNRM, USUHS.
to characterize the extent and time course of TCVI. As novel vascular technologies are developed, we will achieve a better understanding of TCVI's role in clinical symptoms both acutely and remotely after TBI. Understanding the pathophysiology of TCVI will aid in developing effective treatments targeting the underlying pathology.

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