Molecular mechanisms of microvascular failure in central nervous system injury—synergistic roles of NKCC1 and SUR1/TRPM4

A review

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Microvascular failure largely underlies the damaging secondary events that accompany traumatic brain injury (TBI). Changes in capillary permeability result in the extravasation of extracellular fluid, inflammatory cells, and blood, thereby producing cerebral edema, inflammation, and progressive secondary hemorrhage (PSH). Recent work in rat models of TBI and stroke have implicated 2 ion transport proteins expressed in brain endothelial cells as critical mediators of edema formation: the constitutively expressed Na+-K+-2Cl– cotransporter, NKCC1, and the trauma/ischemia-induced SUR1-regulated NCX3-ATP (SUR1/TRPM4) channel. Whereas NKCC1 function requires adenosine 5′-triphosphate (ATP), activation of SUR1/TRPM4 occurs only after ATP depletion. This opposite dependence on intracellular ATP levels implies that one or the other mechanism will activate/deactivate as ATP concentrations rise and fall during periods of ischemia/reperfusion, resulting in continuous edema formation regardless of cellular energy status. Moreover, with critical ATP depletion, sustained opening of SUR1/TRPM4 channels results in the oncotic death of endothelial cells, leading to capillary fragmentation and PSH. Bumetanide and glibenclamide are 2 well-characterized, safe, FDA-approved drugs that inhibit NKCC1 and the SUR1/TRPM4 channel, respectively. When used alone, these drugs have provided documented beneficial effects in animal models of TBI- and ischemia-associated cerebral edema and PSH. Given the mechanistic and temporal differences by which NKCC1 and the SUR1/TRPM4 channel contribute to the pathophysiological mechanisms of these events, combination therapy with bumetanide and glibenclamide may yield critical synergy in preventing injury-associated capillary failure. (DOI: 10.3171/2009.11.JNS081052)

KEY WORDS • traumatic brain injury • ischemia • capillary • NKCC1 • SUR1 • TRPM4

Each year, 1.5 million Americans sustain TBI. As a result 50,000 people die, 230,000 people are hospitalized and survive, and 80,000–90,000 people experience the onset of long-term disability. Traumatic brain injury is the leading cause of death and disability in children and adults between the ages of 1 and 44 years. Overall, more than 5 million Americans—2% of the US population—currently live with disabilities resulting from TBI. The consequences in terms of physical impairments, functional limitations, disabilities, societal restrictions, and economic impact are practically immeasurable. In spite of its importance, there is no effective therapy in clinical use that is specifically directed toward ameliorating secondary brain injury after trauma. An important reason for this unfortunate deficiency in clinical care is an incomplete understanding of cellular and molecular processes that underlie secondary brain injury. One important area of deficiency concerns mechanisms of secondary injury related to microvascular dysfunction; in particular, edema formation and “progressive secondary hemorrhage.”

Early Consequences of TBI: Cerebral Edema and PSH

The pathophysiological origin of TBI is complex and involves multiple injury mechanisms that are spatially and temporally specific. Primary traumatic injury is in-
Roles of NKCC1 and SUR1/TRPM4 in microvascular failure

 invariably complicated by secondary injury, which results in expansion of the original lesion and concomitant worsening of neurological outcome. Mechanisms of secondary injury may include cytotoxic processes, such as excitotoxicity, free radical damage, apoptosis, and inflammation. In addition, secondary injury may result from microvascular dysfunction, including ischemia, edema, and PSH, the latter a phenomenon wherein microvessels gradually lose their structural integrity and become fragmented, resulting in extravasation of blood and the formation of petechial hemorrhages.

Cerebral Edema

Edema complicates virtually all forms of severe CNS injury. The primary mechanical insults of TBI and its secondary metabolic effects (such as ischemia) alter the permeability of the blood-brain barrier, blood-CSF barrier, and neuroglial cell membranes, thereby disrupting fluid and solute homeostasis of the brain. Minor changes in the composition of ions in the brain’s extra- or intracellular fluid can significantly affect the function of neurons, which rely on precise ionic gradients across their plasma membranes to modulate finely tuned changes in membrane potential that underlie neuronal signaling. Because the contents of the brain are confined by the bony calvaria, even small increases in the total volume of intracranial fluid due to edema can exert mechanical forces that disrupt normal anatomical relationships and increase pressure within the skull. As ICP approaches that of the brain capillaries, perfusion is compromised, leading to ischemia and further accumulation of edema fluid. These increases in ICP can result in devastating neurological injury, including infarction, herniation, and death. The degree of cerebral edema after TBI has been classified into 4 categories of severity, based on studies by the NIH’s Traumatic Coma Data Bank.26 Using this classification, the degree of brain edema documented on the first head CT scan obtained after injury has been shown to be highly correlated with patient outcome. Thus, the reduction of cerebral edema after TBI is important, and new pharmacological tools for decreasing it are badly needed.25

Progressive Secondary Hemorrhage

Contusion of the brain often results in the formation of intraparenchymal petechial hemorrhages, which are frequently complicated by “blossoming” or expansion—that is, PSH. A PSH is a key mechanism of secondary injury post-TBI.25,52 Early progressive hemorrhage occurs in almost 50% of head-injured patients, usually following contusion injury, and is associated with elevations in ICP.45,56 Although sometimes erroneously attributed to continued bleeding of microvessels fractured by the original trauma, PSH actually represents a secondary pathological process with specific, well-defined molecular antecedents, as has been shown in SCI.13,42 Progressive secondary hemorrhage occurs during the first several hours after a traumatic insult. It results from progressive catastrophic failure of the structural integrity of capillaries, and is characterized by the formation of small discrete satellite (petechial) hemorrhages in tissues surrounding the site of primary injury. With time, petechial hemorrhages increase in number and eventually coalesce into a hemorrhagic lesion that encompasses the entire site of primary injury. A PSH is particularly damaging because it greatly expands the volume of neural tissue destroyed by the primary injury. The capillary dysfunction underlying PSH also causes tissue ischemia and hypoxia, and the hemorrhage that characterizes PSH is exquisitely toxic to neural tissues,33,54 because it incites free radical formation and inflammatory responses that are especially damaging to the myelin of white matter tracts, thereby worsening the overall neurological injury. Together, these processes render PSH the most destructive mechanism of secondary injury involving the CNS. Whereas ischemia and edema have historically been targeted for treatment, PSH has not—simply because secondary hemorrhage has not previously been viewed as being preventable.

Role of NKCC1 in Edema Formation and Neurotoxicity in TBI and Ischemia

Recent work in rat models of stroke and TBI has implicated 2 ion transport proteins expressed in brain endothelial cells as critical mediators of edema formation and/or PSH: the constitutively expressed, bumetanide-sensitive Na+-K+-2Cl– cotransporter NKCC1, and the ischemia-induced, glibenclamide-sensitive SUR1-regulated NCX–ATP (SUR1/TRPM4) channel.

Derangements in ion transport across brain cell membranes, the blood-brain barrier, and the choroid plexus underlie the formation of cerebral edema. The Na+-K+-2Cl– cotransporter NKCC1 is expressed in glia, cortical and cerebellar neurons, brain capillary endothelial cells, and epithelial cells of the choroid plexus; in these cells, NKCC1 plays an essential role in cell volume regulation and/or transepithelial ion transport. Ischemia-induced isosmotic cell swelling (cytotoxic edema) is a primary driver of cerebral edema, and alterations in transendothelial ion transport underlie ionic and vasogenic edema. Expressed on the luminal side of brain endothelial cells, NKCC1 plays an important role in the formation of ionic edema by loading sodium into endothelial cells.5,12,19,26–30 The sodium inside endothelial capillary cells is then expelled into the brain’s extracellular space by the activity of Na+-K+ ATPase, which is expressed on the abluminal membrane. Trauma and its associated ischemic injury to brain cells, through various mechanisms, has been shown to increase the expression and activity of NKCC1, resulting in impaired cell volume regulation, pathological cell swelling, and the alterations in transendothelial capillary permeability that accompany ionic and vasogenic edema. Genetic deficiency or pharmacological inhibition of NKCC1 with a low dose of the furosemide-related diuretic, bumetanide, significantly reduce cerebral edema and neuronal injury following traumatic and ischemic brain injury.2,5,6,9,12,19,29,30,36,47,48,57

The protein NKCC1 is a cation-chloride cotransporter of the SLC12 gene family; NKCC1 is an intrinsic membrane protein that transports chloride ions, together with sodium and/or potassium ions, across plasma membranes.
of cells. The stoichiometric coupling and directionality of the cations and chloride ions translocated by the NKCC1 results in an electrically silent (that is, electroneutral), secondarily active transport process that is energetically driven by transmembrane sodium and potassium gradients established by Na+-K+ ATPase. Using the large electrochemically favorable inward gradient for sodium ions across the plasma membrane, NKCC1 loads chloride ions into the cell, raising the level of [Cl\(^{-}\)]i above its electrochemical equilibrium. At low concentrations (2–10 \(\mu\)M), bumetanide is a specific inhibitor of NKCC1.

The activity of NKCC1 determines the level of [Cl\(^{-}\)]i. Neurons, glia, endothelial cells, and epithelial cells that line the brain’s ventricular system regulate [Cl\(^{-}\)]i to help maintain their cellular volume amid changes of extracellular osmolality and intracellular solute content, thus preventing the excessive swelling or shrinking that could undermine their structural integrity. Derangements in the expression and activity of NKCC1 can alter [Cl\(^{-}\)]i, disrupting the homeostasis of cell volume and altering the absorption or secretion of ions across epithelia.

The cotransporter NKCC1 contributes to cerebral edema following TBI.\(^{21-23,46}\) A transient, time-dependent upregulation of NKCC1 mRNA and protein was detected in the rodent hippocampus after TBI induced by the calibrated weight drop technique.\(^{21}\) Inhibition of NKCC1 with bumetanide 20 minutes prior to TBI decreased edema and reduced contusion damage.\(^{22}\) This effect of bumetanide was correlated with decreased phosphorylation of Raf/MEK/ERK cascade proteins.\(^{21}\)

Cerebral edema from cold trauma is decreased after administration of torsemide, another NKCC1 inhibitor that can also block chloride channels.\(^{46}\) Although torsemide can result in systemic dehydration that could affect brain edema, in these studies plasma osmolality was not changed, suggesting that torsemide-mediated effects on brain swelling were provided through NKCC1 inhibition. The NKCC1 mRNA and protein levels are also upregulated on the apical membrane of the choroid plexus 2 hours after TBI, with levels peaking at 8 hours and lasting for 24 hours.\(^{22}\) In this study, although rats in the experimental group displayed severe brain edema and contusion volume 8 hours after TBI, administration of bumetanide significantly attenuated contusion volume and brain edema. We have independently confirmed the potent effect of bumetanide in ameliorating edema following TBI (Fig. 1).

Glutamate-mediated neurotoxicity also plays an important role in neuronal damage after traumatic and ischemic injury in the CNS. Acute excitotoxic neurodegeneration after glutamate receptor activation is dependent on sodium and chloride entry.\(^{35}\) Via an unknown mechanism, activation of NMDA and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors stimulates NKCC1 activity in neurons.\(^{36}\) Thus, NKCC1 contributes to the overload of sodium and chloride during glutamate-mediated acute excitotoxicity.\(^{2}\) Blocking NKCC1 activity with bumetanide abolishes glutamate-triggered sodium and chloride accumulation by more than 50%. Oxygen-glucose deprivation–induced neuronal death is also mediated by NMDA ionotropic receptor–triggered excitotoxicity.\(^{14}\) Bumetanide has also been shown to abrogate glutamate and NMDA-mediated excitotoxic cell death. Oxygen-glucose deprivation–mediated cell swelling and death is also significantly attenuated by bumetanide.\(^{5}\)

During ischemia, the level of [K\(^{+}\)]o increases significantly. A few minutes of anoxia/ischemia raises [K\(^{+}\)]o, to ~60 mM. In astrocytes, NKCC1 has been shown to play an important role in potassium uptake under conditions of high [K\(^{+}\)]o. In 75 mM [K\(^{+}\)]o, NKCC1-mediated potassium influx was significantly stimulated in astrocytes; this high [K\(^{+}\)]o-induced activation of NKCC1 is completely abolished by either removal of extracellular calcium or blocking of L-type voltage-dependent calcium channels with nifedipine.\(^{37,48}\) These data suggest that NKCC1 activity is stimulated under high [K\(^{+}\)]o, via calcium-mediated signal transduction pathways. Intracellular accumulation of radiolabeled sodium and chloride is significantly increased in response to 75 mM [K\(^{+}\)]o; this increase is abolished by bumetanide or by genetic ablation of NKCC1. High [K\(^{+}\)]o-mediated stimulation of NKCC1 can result in cell swelling via a net increase of intracellular sodium, potassium, and chloride, and accompanying water. High [K\(^{+}\)]o causes cell swelling in NKCC1\(^{+/+}\) astrocytes, but is absent in NKCC1\(^{-/-}\) astrocytes, and is abolished with bumetanide. High [K\(^{+}\)]o-induced astrocyte swelling is also observed in the rat optic nerve model.\(^{24}\) In enucleated nerves, light transmittance progressively increases with high [K\(^{+}\)]o, causing cell swelling. Bumetanide can reversibly suppress this high [K\(^{+}\)]o-induced cell swelling.

Multiple in vitro and in vivo studies have also shown that NKCC1 plays a role in ischemic cell damage, and that its pharmacological inhibition is neuroprotective.\(^{57}\) Administration of bumetanide either prior to or during ischemia significantly reduces brain edema and infarction after 2-hour MCA occlusion and 24-hour reperfusion in rats. Similar neuroprotection is observed in NKCC1-null mice following MCA occlusion.\(^{6}\) Focal cerebral ischemia leads to increased NKCC1 protein expression in the ipsilateral cortex and striatum after 2-hour ischemia and 24-hour reperfusion in rats.\(^{57}\) In rats subjected to permanent MCA occlusion, intravenous administration of bumetanide immediately before occlusion attenuates edema formation as determined on brain MR imaging.\(^{29,30}\) This further suggests a role for NKCC1 in edema formation during cerebral ischemia.

The SUR1/TRPM4 Channel in Ischemia and TBI

The SUR1-regulated NC\(_{\text{Ca-ATP}}\) (herein called SUR1/TRPM4) channel is a critical mediator of cerebral edema formation. This newly discovered channel is formed from 2 subunits; a regulatory subunit (SUR1) and a pore-forming subunit (TRPM4).\(^{7,13,38,41,43}\) The properties of this channel have been reviewed.\(^{40,41,43}\) It is a 35-pS cation channel that conducts inorganic monovalent cations, but is impermeable to Ca\(^{2+}\) and Mg\(^{2+}\).\(^{9}\) Channel opening requires nanomolar concentrations of Ca\(^{2+}\) on the cytoplasmic side, and is prevented by intracellular ATP (EC\(_{50}\) ~ 1 \(\mu\)M). Although no specific blocker exists for the pore-forming subunit TRPM4, fortunately the receptor-channel complex, SUR1/TRPM4, is blocked with high affinity and specificity by the sulfonyleurea antagonist glibenclamide (EC\(_{50}\) 48 nM).\(^{7}\)
The SUR1/TRPM4 channel is not constitutively expressed, but is expressed in the CNS under conditions of injury or hypoxia. The channel was first discovered in reactive astrocytes obtained from the hypoxic inner zone of the gliotic capsule after stab injury and foreign body implantation. Since then, it has been identified using patch clamp electrophysiological analysis in neurons obtained from the core of an ischemic stroke and in cultured human and mouse endothelial cells subjected to hypoxia. Apart from patch clamp recordings to demonstrate the presence of the channel, CNS tissues have been analyzed to detect the regulatory subunit SUR1 at protein and mRNA levels. Normally, SUR1 is expressed in some neurons, but not in astrocytes or capillaries. Following injury, SUR1 is upregulated in several rodent models of CNS injury, including cerebral ischemia (MCA occlusion), SAH, SCI, and TBI (Simard and colleagues, unpublished). In humans, SUR1 is upregulated in brain parenchyma adjacent to an intracerebral hemorrhage, in germinal matrix of premature infants who sustain or are at risk to sustain germinal matrix hemorrhage, in SCI, and in TBI (Simard and colleagues, unpublished presentation at the Christopher and Dana Reeve Foundation Bi-Annual Meeting, Atlanta, Georgia, 2008). Upregulation of SUR1 is found in all members of the neurovascular unit; that is, neurons, astrocytes, and capillary endothelial cells.

The consequences of opening the SUR1/TRPM4 channel have been studied in cells by depleting ATP to mimic injury conditions. The ATP depletion induces a strong inward current that depolarizes the cell completely to 0 mV. Cells subsequently undergo osmotic swelling (cytotoxic edema). Eventually, ATP depletion leads to cell death, predominantly by nonapoptotic, propidium iodide–positive osmotic (necrotic) cell death. Oncotic cell death is significantly inhibited by glibenclamide.

The SUR1/TRPM4 Channel: Role in Edema Formation in Ischemia and TBI

The effect of blocking with the potent SUR1 inhibitor glibenclamide on edema formation and brain swelling has been studied in 4 rat models of ischemic stroke—malignant cerebral edema, thromboembolic stroke, permanent MCA occlusion, and temporary MCA occlusion with reperfusion. In the malignant cerebral edema model, glibenclamide reduced mortality and cerebral edema by half (Fig. 1). In nonlethal stroke models, glibenclamide reduced lesion volume by half, and its use was associated with cortical sparing that was attributed to improved leptomeningeal collateral blood flow due to reduced mass effect from edema. The therapeutic window for glibenclamide in MCA occlusion extends beyond 6 hours after the onset of ischemia. In a rat model of SAH, the blocking of SUR1 with glibenclamide significantly reduced inflammation, vasogenic edema, and caspase-3 activation.

Ionic Versus Vasogenic Edema

Cerebral edema may be distinguished as “ionic” or “vasogenic” edema, depending on the principal solute that is transported across the capillary barrier (reviewed in Simard et al.). An excess of sodium-rich, protein-poor fluid is referred to as ionic edema, whereas protein-rich fluid is referred to as vasogenic edema. The formation of ionic edema is believed to be due to transcellular flux of sodium across the endothelial cell barrier, which, as mentioned previously, can be due to activity of NKCC1/Na"K+ ATPase or to SUR1/TRPM4 (Fig. 2). In TBI, ionic edema may be more important than vasogenic edema.

The formation of vasogenic edema is more complex and requires the introduction of additional cellular elements. Dynamic control of the endothelial barrier in-

![Fig. 1. Bar graphs showing that bumetanide and glibenclamide reduce edema formation. Brain water content in rodent models of TBI (left) and cerebral ischemia (right), without (VEH) and with treatment using bumetanide (BUM) (left) or glibenclamide (GLIB) (right). The TBI was induced by cortical impact (2.5–3 atm) following craniectomy to expose the dura mater; bumetanide (15 mg/kg given intraperitoneally, plus 200 µg/hour given subcutaneously via miniosmotic pump) was administered starting within a few minutes after injury, and tissue water was determined at 6 hours (JM Simard and V Gerzanich, unpublished data). Ischemia was induced by permanent occlusion of the MCA; glibenclamide (75 ng/hour, given subcutaneously via miniosmotic pump) was administered starting within a few minutes after injury, and tissue water was determined at 8 hours (Simard et al. ). Brain water was determined using the wet weight/dry weight method; 5 rats were used per group. **p < 0.01.](https://example.com/figure1.png)
volves signaling between the endothelial cytoskeleton and the adhesion complexes between neighboring cells.\textsuperscript{18,51} The actual barrier is made up of the physical elements of tight junction complexes, the major constituents of which include transmembrane (junctional adhesion molecule–1, occludin, and claudins) and cytoplasmic (ZO-1 and -2, cingulin, AF-6, and 7H6) proteins linked to the actin cytoskeleton of endothelial cells.\textsuperscript{15,51} The proper cellular location and sealing of interendothelial tight junctions depends on the scaffolding properties of ZO-1 and its relationship to the actin cytoskeleton.\textsuperscript{11,18} Injury or insult causes rearrangement of the cytoskeleton, ZO-1 disruption, cell retraction, and the formation of intercellular gaps,\textsuperscript{3,11,55} which allow paracellular flow of protein-rich plasma, also known as the formation of vasogenic edema (Fig. 2).

In many cells, an increase in sodium concentration or a perturbation of cell volume leads to reorganization of the actin cytoskeleton and weakening of intercellular tight junctions, leading in turn to an increase in barrier permeability.\textsuperscript{32} This same phenomenon is believed to occur in brain endothelial cells in which SUR1/TRPM4 channels are activated. Upregulation and activation of SUR1/TRPM4 channels leads to excess sodium influx and cytotoxic edema of endothelial cells,\textsuperscript{7,8,42} providing a plausible molecular mechanism to account for the cytoskeletal rearrangement that leads to the formation of vasogenic edema. We recently reported the redistribution of ZO-1 in the endothelium of the posterior cerebral artery in association with the formation of vasogenic edema following SAH, along with concomitant reduction in these abnormalities by SUR1 inhibition with glibenclamide.\textsuperscript{39} These findings are consistent with the hypothesis that vasogenic edema is tied to a significant disruption of the endothelial actin cytoskeleton. The conceptualization outlined here provides a coherent picture linking an insult that results in upregulation and opening of SUR1/TRPM4 channels in endothelial cells to sodium influx, endothelial cell swelling and actin cytoskeletal rearrangement, loss of endothelial tight junction integrity, and formation of vasogenic edema.

The SUR1/TRPM4 Channel: Role in PSH in TBI and Ischemia

Apart from its important role in the formation of edema, the SUR1/TRPM4 channel is also critically involved in PSH.\textsuperscript{13,42} When ATP is depleted to critical levels for sustained periods of time, unopposed opening of SUR1/TRPM4 channels results eventually in oncotic death of endothelial cells, leading to capillary fragmentation and PSH, resulting in even greater tissue destruction. This mechanism of secondary hemorrhage was first discovered during study of a rodent model of SCI. An SCI results in PSH, which is characterized by a progressively expansive lesion with fragmentation of capillaries, hemorrhage that
Roles of NKCC1 and SUR1/TRPM4 in microvascular failure

doubles in volume over 12 hours, tissue necrosis, and severe neurological dysfunction. Necrotic lesions are surrounded by widespread upregulation of SUR1 in capillaries and neurons. Following SCI, blocking of SUR1 by glibenclamide essentially eliminates capillary fragmentation and PSH, is associated with a 3-fold reduction in lesion volume, and results in marked neurobehavioral functional improvement.

The ATP Requirements of NKCC1/Na+K+ ATPase Versus the SUR1/TRPM4 Channel—Synergy in Molecular Mechanisms

It is particularly noteworthy that the 2 molecular mechanisms of ionic edema formation involving NKCC1/Na+K+ ATPase and the SUR1/TRPM4 channel exhibit opposite dependencies on the cellular concentration of ATP (Fig. 2).

The NKCC1 cotransporter, expressed on the luminal side of the endothelium, loads sodium into endothelial cells,[5,12,19,29,30] which then expel the sodium into the brain’s extracellular space by the activity of abluminal Na+K+ ATPase. Because of the obligatory requirement for ATP for functional activation of Na+K+ ATPase, the contribution of NKCC1/Na+K+ ATPase to ionic edema is important, both in the early stages of ischemia and during reperfusion, at times when endothelial cells have adequate levels of ATP to drive the activity of the Na+K+ ATPase.

Conversely, the SUR1/TRPM4 channel, which is expressed on both the luminal and abluminal membranes, is inactive when ATP levels are adequate. The channel opens only when ATP levels are significantly decreased (EC_{50} ~ 1 \mu M at neutral pH), and when opened on both luminal and abluminal sides, it facilitates transcapillary flux of sodium from blood to the brain’s extracellular space, which in turn drives the formation of ionic edema.[20]

This opposite dependence on cellular ATP suggests that one or the other mechanism will activate/deactivate as ATP concentrations rise and fall during periods of ischemia/reperfusion, thereby resulting in continuous formation of ionic edema, regardless of energy status. When ATP levels are adequate, NKCC1/Na+K+ ATPase will be active and SUR1/TRPM4 will be blocked; when ATP levels are low, NKCC1/Na+K+ ATPase will be inactive and SUR1/TRPM4 will become active (Fig. 2). Given this framework, inhibiting one and not the other will yield incomplete inhibition of edema formation (Fig. 1), whereas inhibiting both simultaneously is expected to yield strong synergy in combating the formation of edema.

**Bumetanide and Glibenclamide—Synergetic Combination Therapy**

Given their prominent role in the microvascular failure that largely underlies the cerebral edema and PSH resulting from traumatic and ischemic brain injury, a novel therapeutic combination of drugs targeting NKCC1 and the SUR1/TRPM4 channel would be expected to yield critical synergy in preventing capillary failure, optimizing microvascular circulation, maximizing tissue preservation, and thereby improving functional outcome. Bumetanide and glibenclamide are 2 FDA-approved drugs with strong neuroprotective potential that inhibit NKCC1 and the SUR1/TRPM4 channel, respectively.

Bumetanide is a loop diuretic of the sulfamyl category used to treat heart failure. Experience with its use in humans over 3 decades indicates that it is safe, effective, and well tolerated when taken over a long period,[1,49] suggesting that short-term use postinjury would also be safe and well tolerated. Bumetanide is a relatively specific inhibitor of NKCC1 at low concentrations (~2–10 \mu M). There are no published studies of bumetanide accumulation in the CNS after systemic administration, but the drug’s favorable lipid/water partition coefficient, along with its documented effects on cerebral edema and seizures in vivo in animals and humans, suggest that bumetanide can cross the blood-brain barrier sufficiently to inhibit NKCC1 in the brain. Pilot studies are now underway to assess the efficacy of bumetanide, administered with phenobarbital, for the treatment of another neurological condition, neonatal seizures (FDA IND #101690; see http://www.cureepilepsy.org/research/current.asp [accessed December 10, 2009]).

Glibenclamide (also known as glyburide), a sulfonylurea used to treat Type II diabetes, is 1 of 2 oral antidiabetic agents in the WHO Model List of Essential Medicines. Experience with its use in humans over 4 decades indicates that it is safe, effective, and well tolerated when taken over a long period,[27,24] suggesting that short-term use postinjury would also be safe and well tolerated. A recent analysis of patients with diabetes mellitus who were hospitalized within 24 hours of the onset of acute ischemic stroke was performed to determine whether concurrent use of sulfonylureas might affect stroke outcome.[17] The study compared 33 patients taking a sulfonylurea drug (for example, glibenclamide) at admission through discharge (treatment group) and 28 patients who were not receiving a sulfonylurea drug (control group). The primary outcome, a decrease in the NIH Stroke Scale score of 4 points or more between admission and discharge, or an NIH Stroke Scale score of 0 (major neurological improvement) at discharge, was reached by 36% of patients in the treatment group and by 28% in the control group (p = 0.007), findings consistent with significant neuroprotection.

**Conclusions**

New understanding of the fundamental molecular mechanisms responsible for microvascular failure in CNS ischemia and trauma provides novel insights into genuinely new therapeutic approaches. Given the mechanistic and temporal differences by which NKCC1 and the SUR1/TRPM4 channel contribute to the pathophysiological origins of secondary injury, it is likely that combination therapy with agents such as bumetanide plus glibenclamide will yield critical synergy in preventing capillary failure—thereby optimizing microvascular circulation, maximizing tissue preservation, and improving functional outcome after CNS ischemia and trauma.

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J. M. Simard, K. T. Kahle, and V. Gerzanich
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