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REVIEW PAPER

Acid–Base and Electrolyte Changes Drive Early Pathology in Ischemic Stroke

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Abstract

Emergent large vessel occlusion accounts for 20–40% of ischemic strokes and is the most debilitating form of stroke. Some of the earliest changes in response to ischemic stroke occur in blood gases and electrolytes. These biochemical changes occur within minutes after occlusion in experimental models of stroke and can be utilized to predict stroke outcomes. The majority of ELVO stroke patients are middle-aged to elderly and are of both sexes, revealing that there is an age and sex mismatch between ischemic stroke patients and animal models, since most experimental studies use young male rats. Rethinking of the animal models should be considered, especially in encouraging the use of aged male and female rats with comorbidities to more closely mirror human populations. Mechanical thrombectomy provides a unique opportunity for researchers to further this work by expanding the collection and analysis of blood samples that are adjacent to the thrombus. To understand the complexity of stroke, researchers can analyze these tissues for different molecular targets that occur in response to ischemic stroke. This information may aid in the reduction of symptom burden for individuals diagnosed with ischemic stroke. Investigators should also focus on data from ischemic stroke patients and attempt to discover target molecules and then in animal models to establish mechanism, which will aid in the development of new stroke therapies. This review discusses the translation of these studies to the human patient to develop the capability to predict stroke outcomes. Future studies are needed to identify molecular targets to predict the risk of worsened long-term outcomes and/or increased risk for mortality.

Keywords Brain ischemia · Blood gases · Electrolytes · pH

Ischemic stroke is the leading cause of disability in the United States, and affects approximately 800,000 Americans per year (Benjamin et al. 2019). It is the third leading cause of death for women, occuring in about 55,000 more women than men each year (Benjamin et al. 2019). African Americans have twice the risk of having a first stroke when compared with Caucasians, in addition to an increased mortality rate from stroke (Benjamin et al. 2019). In Kentucky, stroke is the fifth leading cause of mortality (CDC 2006). Given the pervasiveness of stroke, it is critical to develop a more complete understanding of the pathophysiology at the time of stroke onset; these insights may reveal potential targets for stroke treatment.

The majority of strokes (87%) are ischemic and result from a thrombus obstructing a major artery in the brain (Go et al. 2014). The blockage caused by the thrombus produces initial hypoxic damage that is followed by ischemia and inflammation (Doyle et al. 2008; Tobin et al. 2014). Traditionally, the 'at risk' area has been delineated into two zones. As neurons die, they produce an infarct core. The penumbra, the area surrounding the core, also includes tissue surviving but at risk of further death; the core may expand to this area if reperfusion does not occur (Borgens et al. 2012). Shifts occur within the cerebral blood flow (CBF) leading to disturbances in brain metabolism. Disruption in blood flow reduces oxygen (O_2) and glucose, and leads a mismatch in

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demand and production of adenosine triphosphate (ATP) (Kristian et al. 1997). Oxygen availability to the brain is the product of CBF and the O_2 content of arterial blood. Cerebral ischemia occurs when metabolic demand for O_2 is not met.

The presence of the thrombus decreases CBF resulting in acidosis due to a lack of glucose, production of lactate, and an increase in carbon dioxide (CO_2). A lack of glucose triggers cellular glycolysis increasing of lactic acid production and a lowering in pH disrupting normal acid/base balance within the brain (Back et al. 2000; Casey et al. 2010). During ischemia, CO_2 from neural cells accumulate in intracellular spaces, which lead to a further reduction in pH levels (Traystman et al. 1985).

When blood pH decreases, electrolyte concentrations of sodium (Na^+), calcium (Ca^{2+}), and potassium (K^+), which maintain cellular structure and function, are affected (Kristian et al. 1997). Dysregulation of ion pumps (Na^+/K^+ and Na^+/Ca^{2+}) occurs, causing irregularities in trans-membrane gradients among cells. Under normal conditions Na^+ is pumped out of the cell and K^+ into the cell, but without a supply of ATP, the pump stops working properly. The increased concentration of intracellular Na^+ leads to cytogenic edema. The disruption of the Na^+/Ca^{2+} pump leads to an increased concentration of intracellular Ca^{2+}, which is detrimental in several ways (Mifsud et al. 2014). It causes excitotoxicity by the release of glutamate neurotransmitters, which spurs neighboring neurons to also become overexcited. It also activates lipases that break down neuron cell membranes allowing other ions to enter the neuron. Finally, this process produces an increase of cytoplasmic calcium causing mitochondrial dysfunction, leading to the generation of free radicals and reactive oxygen species (ROS) that are responsible for neural cell death (Mifsud et al. 2014).

These initial pathophysiological changes can produce irreversible cell death. Thus, time is critical in the treatment of stroke to minimize and reverse this process. Current treatments pharmacologically break up (via tissue plasminogen activator [tPA]) or physically remove the thrombus (via mechanical thrombectomy) (Tissue plasminogen activator for acute ischemic stroke 1995; Berkhemer et al. 2015; Goyal et al. 2016). tPA catalyzes the conversion of plasminogen to plasmin and is the primary mechanism for thrombolysis. The use of intravascular tPA is time dependent; due primarily to the risks of hemorrhage at later time points, patients can only be administered tPA within 4.5 h after their last known normal time (LKN—the time prior to stroke onset at which the patient was last known to be without stroke signs and symptoms). Mechanical thrombectomy is an intraarterial procedure during which the thrombus is directly removed and blood flow is re-established. It is approved and standard of care for the treatment of emergent large vessel occlusions (ELVO) (Fiorella et al. 2015; Campbell et al. 2015). ELVO is an occlusion of one of the main arterial vessels of the Circle of Willis (Leslie-Mazwi et al. 2018). Recently, the American Heart Association/American Stroke Association changed the time window for mechanical thrombectomy; with multiple randomized clinical trials showing benefit, mechanical thrombectomy is now a standard of care procedure recommended for anterior circulation ELVO up to 24 h in selected cases (Ackerson 2018; Nogueira et al. 2018; Albers et al. 2018).

Interestingly, thombectomy has also provided researchers with a rare opportunity to examine neurochemical changes that occur during stroke. It allows researchers to isolate the clot, and, in some cases, blood within the artery immediately distal and proximal to the thrombus (systemic arterial blood in the cervical carotid artery) (Sporns et al. 2017; Maekawa et al. 2018; Prochazka et al. 2018; Fraser et al. 2019;). The analysis of blood samples from the injury site allows for a systematic understanding of ischemic stroke neuropathology at the time of mechanical thrombectomy post-stroke. The analysis of these blood samples may help us better understand the sources of thrombus, early neurochemical changes after stroke onset, interaction in different pathways of injury, and responsiveness to treatment. Furthermore, understanding molecular mechanisms of injury at the site may assist in the development of adjunctive therapies to compliment current stroke treatments. This information may aid in the reduction of symptom burden for individuals diagnosed with ischemic stroke.

Acid/Base Balance and Electrolyte Concentrations in Large Vessel Occlusion

Researchers have examined the relationship of venous and arterial blood gas parameters in critically ill patients (Kelly et al. 2001; Ak et al. 2006; Khan et al. 2010; Treger et al. 2010; Awasthi et al. 2013; Esmaeilivand et al. 2017) and in rodent models (Son et al. 2010; Schwarzkopf et al. 2013). These studies demonstrate that venous blood gas values in rodents and humans are similar and therefore comparable. Flores et al. (2013) examined arterial blood gases and electrolyte concentrations in distal blood adjacent to the cerebral thrombus and peripheral blood from the carotid artery during mechanical thrombectomy of ischemic stroke patients ($n = 16$), with results indicating significant differences between the two blood samples in partial pressure oxygen (distal 73.9 ± 14.9 and proximal 78.9 ± 16.3, $p = 0.007$) and oxygen saturation (distal 93.2 ± 4.4 and proximal 94.3 ± 3.9, $p = 0.003$) (Flores et al. 2013). Back et al. (2000) assessed pH regulation in rodents within 30 min and 6 h of permanent middle cerebral artery occlusion (MCAO) model. They found acidosis (pH 6.03) in the infarct core and alkalosis (pH 7.32) in the penumbral area (Back et al. 2000). Together,

these studies provide initial insights into acid/base balance and electrolyte concentrations. The gap in the knowledge is that we do not have blood gas values at baseline or stroke onset in large vessel occlusion.

Researchers have also examined peripheral blood electrolyte changes that occur after stroke. A lowering in total serum calcium concentrations in peripheral venous blood in stroke patients were associated with more severe clinical symptoms following stroke onset, including worse functional outcomes, and an ishemic stroke to hemorrhagic conversion after thrombectomy (Guven et al. 2011; Guo et al. 2015; Ishfaq et al. 2017). Higher total serum calcium levels from peripheral venous blood were detected on patient admission or within 24 h of stroke onset were associated with improved functional outcomes and decreased infarct volumes (Ovbiagele et al. 2006; Buck et al. 2007; Ovbiagele et al. 2008). Serum Na^+ levels from peripheral venous samples were associated with stroke severity but the results are inconclusive. Rodrigues et al. (2014) and Soiza et al. (2015) found patients' with lower sodium levels or hyponatremia were associated with increased stroke severity and risk for mortality. Others have observed higher venous serum sodium concentrations were associated with stroke incidence and neurological worsening (Christensen et al. 2002; Fofi et al. 2012; Farahmand et al. 2013). Serum Na^+ levels in the control group of healthy individuals were lower than in patients with transient ischemic attack and with ischemic and hemorrhagic strokes (Grotta et al. 1982).

Early changes in blood gases and electrolytes are some of the first biochemical responses to this ischemic event (Kim et al. 2016) and have the potential to be used as predictors of stroke outcomes and determine directions for therapeutic approaches. However, due to the complexity of the stroke pathology, many factors are involved in the progression of this injury. Our laboratory has utilized statistical techniques to examine these early changes in blood gases and electrolytes to predict different outcomes in rodent models of stroke.

We have reported that changes in venous blood gases occur as early as 7 min after experimental ischemia and these changes predict stroke outcomes in young males and aged rats of both sexes (Martha et al. 2018, 2019). To date, no other study has examined acid/base balance and electrolyte changes this early after permanent middle cerebral artery occlusion (MCAO) and transient MCAO rat models or examined these changes in aged male and female rats. The MCAO model is similar emergent large vessel occlusion (ELVO) in human stroke patients, which accounts for 20–40% of ischemic strokes and is the most disabling form of stroke (Go et al. 2014; Fiorella et al. 2015; Campbell et al. 2015). These animal models mirror two stroke situations—a patient receiving stroke treatments, such as thrombectomy or tissue plasminogen activator (transient model) and the natural history of a stroke patient who does not receive treatment (permanent model). In addition, this is the first time these changes were tested as predictors of infarct volume, edema volume, and/or mortality in rats.

Our first report assessed acid/base balance and electrolyte concentrations changes in the jugular venous blood in both permanent MCAO and transient MCAO stroke models using young (3-month-old) male rats. Additionally, there is no existing literature that evaluates early acid/base and electrolytes in focal ischemia and uses these values for predictors of stroke outcomes (infarct volume and/or mortality). Data from these models were analyzed using paired samples t-tests, Kaplan–Meier survival curve, and multiple linear regression analyses. Pre- and post-MCAO venous blood samples from permanent and transient models provided pH, carbon dioxide (CO_2), oxygen (O_2), bicarbonate (HCO_3^-), glucose, hemoglobin, hematocrit, and electrolyte values of ionized calcium (iCa^{2+}), potassium (K^+), and sodium (Na^+). Mean differences were seen in the blood gas and electrolyte concentrations between pre- to post-MCAO in both models. The pH and iCa^{2+} were predictors of infarct volume in the permanent MCAO model but not in the transient model. As expected, the transient MCAO model exhibited greater survival to 72 h than the permanent MCAO model (Martha et al. 2018).

This same paradigm depicted acid/base balance and electrolyte changes occurring within 7 min in the permanent MCAO model in aged (18-month-old) male and female rats. Most stroke studies use young male rats as their population, and this does not reflect the age or sex balance of the patient population that suffers from strokes. This study used aged male and female rats to evaluate early acid/base and electrolyte to determine predictors of infarct volume, edema volume, and mortality. Data from these models were analyzed using a one-way repeated measures ANOVA, multiple linear regression, and Cox regression analyses which compared acid/base balance and electrolyte concentrations at three time points between aged male and female rats (Martha et al. 2019). Venous blood gas samples of aged Sprague–Dawley male and female rats were examined at (1) pre-, (2) post-, and (3) at 72 h. The repeated measures ANOVA revealed no mean differences in acid/base and electrolyte concentrations from the three time points between the sexes. However, changes in pH (from pre- to post-MCAO and post-MCAO to 72 h) and changes in Na^+ and iCa^{2+} (from post-MCAO to 72 h) were predictors of infarct volume and edema volume, respectively. There was a 3.25 times increased risk for mortality in rats based on changes (cut-off range within -2.00 to −7.00) in HCO_3^- levels (pre- to post-MCAO).

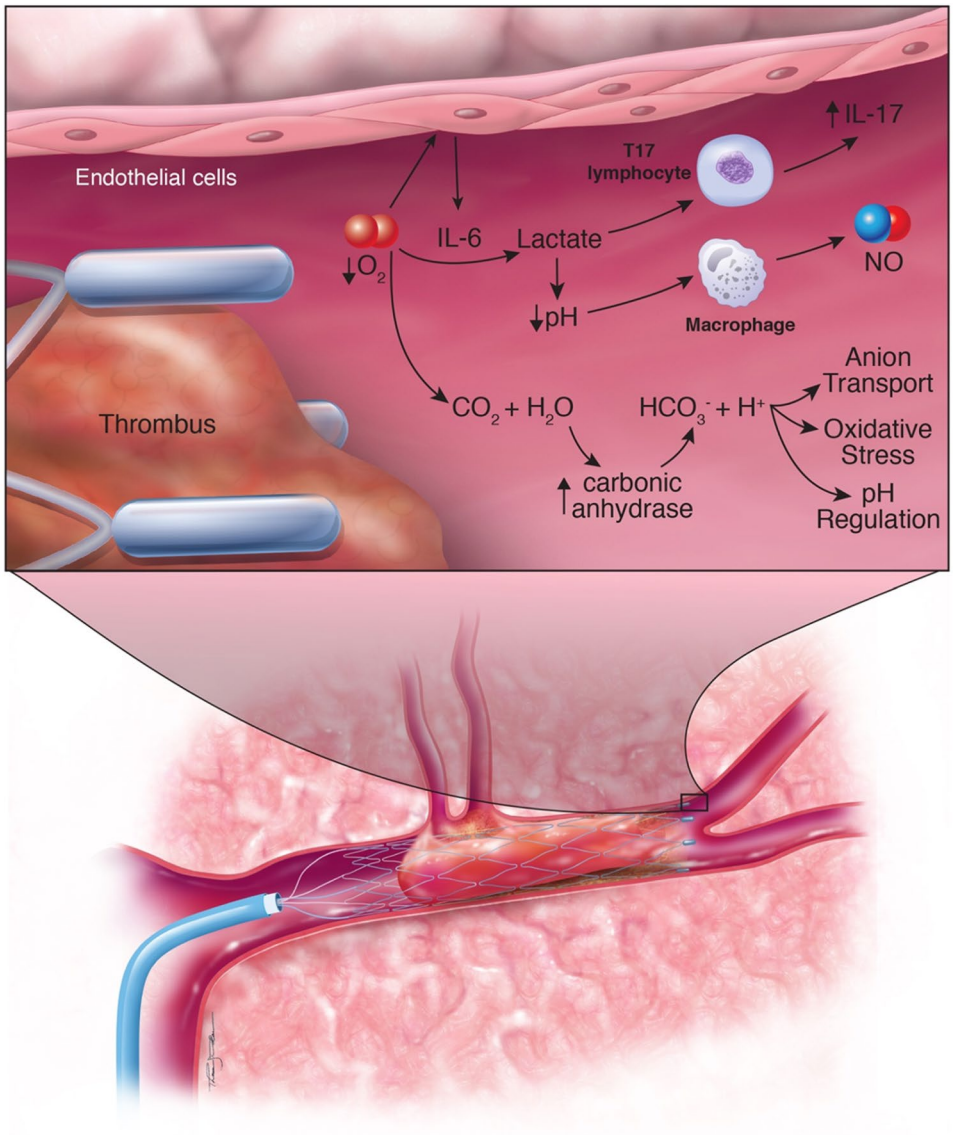

Fig. 1 Illustration of some anticipated mechanisms for relationship of acid/base balance and neuroinflammatory cascade. These studies will aid in mapping these cascades in the human condition

Ramifications of Blood Gas and Electrolyte Changes

These changes in blood gases and electrolytes are caused by the dysregulation of acid/base homeostasis from blockage of blood flow during ischemia. Bicarbonate, H^+, and CO_2 provide a dynamic buffering system to regulate pH homeostasis, and to facilitate anion/water transport (Hamm et al. 2015). Inadequate ATP supply leads to sodium and calcium to enter the cells which results in malignant cerebral edema. In myocardial ischemia/reperfusion injury, the presence of bicarbonate causes increased tissue oxidative damage, which leads to increased inflammation (Queliconi et al. 2013). ELVO can be life threatening with an associated increased intracranial pressure leading to malignant cerebral edema resulting in cerebral herniation and death (Mokri et al. 2001; Simard et al. 2007). Moreover, the decreased oxygen leads to increased lactate in this environment, which has been shown to contribute to local inflammation (Fig. 1). For example, hypoxia leads to endothelial cells releasing the inflammatory cytokine, IL-6, into the blood to induce an inflammatory Th17 phenotype of T helper cells (Yan et al. 1995). The increased levels of lactate directly produce the highly inflammatory IL-17 cytokine (Haas et al. 2015). This local environment of low pH induces macrophages to produce nitric oxide, which causes oxidative damage to cells (Bellocq et al. 1998). These are but a few examples that link initial changes in blood chemistry to an inflammatory cascade that lasts days after the stroke.

Studies are needed to link the basic rodent research with human stroke patients. The animal work does show that blood chemistry changes very rapidly in response to blockage of blood of flow. With the continuous increase in mechanical thrombectomy utilization, there is a growing opportunity to bring the ‘bench to the bedside’. We are

currently using the mechanical thrombectomy procedure to collect and study blood samples from the area of the infarct from patients undergoing stroke (Fraser et al. 2019). Preliminary data from these efforts show bicarbonate and other electrolytes responding to ischemic stroke in human patients (data unpublished). Thus, early biochemical changes have a profound effect on stroke pathology and warrant more in-depth evaluation.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. (IACUC 2016-2356).

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