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Early Acid/Base and Electrolyte Changes in Permanent Middle Cerebral Artery Occlusion: Aged Male and Female Rats

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Abstract

Background: Early changes in acid/base and electrolyte concentrations could provide insights into the development of neuropathology at the onset of stroke. We evaluated associations between acid/base and electrolyte concentrations, and outcomes in permanent middle cerebral artery occlusion (pMCAO) model.

Methods: 18-month old male and female Sprague-Dawley rats underwent pMCAO. Pre-, post- (7 minutes after occlusion), and at 72 hours of pMCAO venous blood samples provided pH, carbon dioxide, oxygen, glucose, hematocrit, hemoglobin, and electrolyte values of ionized calcium, potassium and sodium. Multiple linear regression determined predictors of infarct and edema volumes from these values, Kaplan-Meier curve analyzed morality between males and females at 72 hours, and a Cox regression model was used to determine predictors for mortality.

Results: Analysis indicated significant differences in acid/base balance and electrolyte levels in aged rats not dependent on sex between the three time points in the pMCAO model. Changes in

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The datasets analyzed in the current study are available from the corresponding author upon reasonable request.

pH (from pre- to post and post- to 72 hours) and changes in sodium and ionized calcium (from post- to 72 hours) were predictors of infarct volume and edema volume, respectively. Cox Regression revealed there is a 3.25 times increased risk for mortality based on changes in bicarbonate (pre- to post-MCAO).

Conclusions: These early venous blood changes in acid/base balance and electrolytes can be used to predict stroke outcomes in our rat model of stroke. This study provides potential biomarkers to be examined in the human condition that could provide profound prognostic tools for stroke patients.

Keywords

acid base; electrolytes; aged male/female rats; stroke; infarct/edema volume

INTRODUCTION

Stroke remains the leading cause of death and disability worldwide (Go et al., 2014). Edema and electrolyte imbalance contribute to brain swelling, dysfunction, and death. Currently, there are no early serum tests to predict risk of death from stroke-induced edema. While MRI will show large infarcts early, it is no longer typically used in the emergent presentation due to delaying urgent treatment. Therefore, identifying an easily obtainable clinical marker to predict cerebral edema and death would be a major advancement in stroke care. Many strokes are emergent large vessel occlusion (ELVO), in which there is an acute occlusion of one of the vessels of the Circle of Willis. Accounting for 20–40% of ischemic strokes, it is the most disabling form (Smith et al., 2009). The permanent MCAO model best represents the natural history of emergent large vessel occlusion (ELVO) stroke that is untreated with thrombolytic therapy or mechanical thrombectomy (McBride & Zhang, 2017). Most patients with ELVO currently do not receive either of these treatments, which results in a high mortality rates or severe disabilities (Bhole et al., 2017; Messe et al., 2016).

Blockage of the large vessel artery reduces cerebral blood flow (CBF) below functional levels depriving neurons of oxygenated glucose and blood. The penumbra, the brain area adjacent to the infarction, is at risk of infarction if reperfusion does not occur (Borgens & Liu- Snyder, 2012). Oxygen (O_2) and glucose are reduced in the blood downstream of the occlusion. A deprivation in O_2 and glucose contribute to the failure of adenosine triphosphate (ATP) production and cerebral ischemia occurs when O_2 supply fails to meet metabolic demand (Kristian & Siesjo, 1997). Cells are forced into anaerobic glycolysis which leads to lactic acid accumulation, which lowers the pH (Back et al., 2000; Casey, Grinstein, & Orlowski, 2010). Carbon dioxide (CO_2) builds up and accumulates in extracellular and intracellular spaces leading to decrease in pH (Traystman, Kirsch, & Koehler, 1991). The acidic pH impacts electrolyte concentrations such as sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) which regulate cellular structure and function (Kristian & Siesjo, 1997). Increased concentration of intracellular Na^+ causes cytotoxic edema. Additionally, the disruption of the Na^+/Ca^{2+} pump results in an increased concentration of intracellular Ca^{2+} which initiates apoptosis, causes mitochondrial dysfunction, and generates free radicals and reactive oxygen species (ROS) (Mifsud, Zammit, Muscat, Di Giovanni, & Valentino, 2014). These acid/base and electrolyte changes are associated to the severity of

the stroke (Martha et al., 2018), a greater understanding of the blood gas parameters allows us further insight into how these factors influence stroke outcomes.

Previously, we have reported a change in blood gases and electrolytes within a few minutes of focal ischemia in young male rats and these changes predict infarct volume (Martha et al., 2018). This study uses aged male and female rodents that are better aged-matched to the typical age of the human stroke patient. Therefore, we aimed to examine venous blood gas values between male and female aged rats at three time points: (1) pre-permanent middle cerebral artery occlusion (pMCAO), (2) post-pMCAO, and (3) at 72 hours before euthanization; and to determine whether venous blood gas values predict infarct volume, edema volume, and/or mortality in pMCAO in aged male and female rats. This study identifies early changes in blood gases/electrolytes that predict stroke outcomes in an experimental rodent model of stroke.

MATERIALS and METHODS

Ethics Approval and Animals

Aged male and female rats (18-month old Sprague-Dawley rats (ENVIGO, Indianapolis, IN) were used for all procedures. The aged female rats on average weighed between 245 and 425 grams, and aged male rats approximately weighed between 505 to 705 grams. The study was conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and study protocols were approved by University of Kentucky's (UK) Institutional Animal Care and Use Committee (IACUC). Animals were housed in a climate controlled room on a 12-hour light and dark cycle (0700 – 1900) with access to food and water. Per Division of Laboratory Animal Resources (DLAR) cage requirements at UK's vivarium facility, the animals can be paired in one cage if the animal weight is under 650 grams. We typically house two animals (males or females) per cage upon arrival to DLAR. Once the rats are over 650 grams, they are then split into a separate cage by themselves. Animals were administered vehicle of sterile filtered phosphate buffered saline (PBS) of pH 7.4, at 6, 24, 48, and 72 hours after permanent middle cerebral occlusion (pMCAO). The rats that survived to 72 hours (n = 16) underwent Magnetic Resonance Imaging (MRI) for Diffusion Tensor Imaging (DTI) images and T2 weighted images to measure infarct and edema volumes and then euthanized.

Permanent Middle Cerebral Artery Occlusion

During the pMCAO procedure, as previously described, (Ajmo et al., 2008) animals were placed in an induction chamber and anesthetized with 5% isofluorane/oxygen. During the procedure, a constant flow of 3% isofluorane in 100% oxygen at a rate of 1 L/min was maintained. A midline vertical neck incision occurred to collect an internal jugular blood draw. Glass rods were used to separate the large muscle pad and the sternocleidomastoid muscle (SCM). Retractors were placed to pull musculature of the SCM and skin tightly away from the targeted area. Dissection of the omohyoid muscle, fat, and connective tissues were made to isolate and expose the right common carotid artery from the vagus nerve. Blunt dissection with glass rods occurred around the internal carotid artery (ICA) with removal of lymph nodes and fat surrounding the internal/external cerebral artery (ICA/ECA)

bifurcation to expose the pterygopalatine/middle cerebral artery (MCA) bifurcation. The first clamp was placed on the ICA prior to the pterygopalatine/MCA bifurcation, while the second clamp was placed on the common carotid artery (CCA). The placement of the second clamp further in the posterior direction allows more room for manipulation during the embolus insertion. The ECA was isolated, and used to access the arterial system. A 40mm nylon monofilament was fed distally into the ECA and advanced through to the ICA at approximately 25mm until it reached the ICA clamp. The monofilament was loosely sutured in place, the first clamp was removed, and the embolus was advanced then sutured in place to obtain permanent occlusion of the middle cerebral artery (MCA), M1 segment. The second clamp was removed with careful observation of minimal blood, then the removal of the retractors. Before the neck incision closure, a post- MCAO blood draw occurred from the internal jugular vein.

Post-Surgical Fluid Management and Pain Control

Immediately post-operatively the animals received 2ml of sterile saline (0.9%) subcutaneous. An additional 1ml of saline will be given if extra blood loss occurred during surgery. The animals were scheduled to be injected with sterile filtered PBS pH 7.4 at 6, 24, 48, and 72 hours post-MCAO. The animals are weighed every morning post-MCAO to determine dehydration. Hydration status was checked by pinching up or “tenting” the skin over the nape of the neck. The skin should immediately relax into its normal position. If the skin remains tented longer than normal, the rat was deemed dehydrated and saline was given. Per DLAR guidelines, rats can receive up to 10ml at a time and no more than 2ml at any one location per 6 hours. If warranted, additional saline (1–2ml) will be given in addition to 6, 24, 48, and 72 hours. Also, we add an additional water bottle in each cage to allow more availability to free water for the rats to consume and moist food is provided to encourage feeding and additional water intake.

Post-surgical pain control is managed with carprofen, which is based on weight of the animal. Animal weights are taken prior to surgery (pMCAO) and daily until animals are euthanized at 72 hours (post MRI). The animals receive dosage of carprofen 5mg/kg prior to surgery and every 24 hours for three days post-pMCAO until 72 hours when they are euthanized (post MRI).

Termination of survival criteria include that all animals will be weighed and monitored, especially for dehydration and pain, each morning post surgery. This includes specific attention to surgery and injection sights. If symptoms such as pain, fatigue, loss of energy, excess energy, ruffled hair coat, reluctance to move, failure to groom or feed, hypoactivity, hyperactivity, restlessness, self-trauma, aggressiveness, ataxia, pale mucous membranes, cyanosis, rapid, shallow and/or labored breathing, cachexia, porphyria, soiled anogenital area, inactivity, failure to respond to stimuli, lack of inquisitiveness, vocalization, and/or hunched posture are observed, the research team will obtain advice from the vivarium veterinary staff on how best to intervene to alleviate discomfort; if that is not possible the animal will be euthanatized. Additional checks are made in the afternoon if there is any rat of concern.

The animal will be removed from the study if adverse signs persist despite carprofen and treatment past 24 hours. If the signs fail to resolve, the vivarium veterinarian will be consulted and decide the time course when such animals will be euthanized. Additionally, weight loss greater than 20% (emaciated appearance, rapid weight loss over two days) is considered an endpoint. Rapid weight loss is considered greater than 10% a day for two days.

Venous Blood Gas Collection

Blood gas samples were collected and analyzed pre- and post-MCAO, and prior to euthanization at 72 hours. An internal jugular line was inserted, prior to MCAO monofilament placement and an approximately 100μl of venous blood sample was collected (representing the pre-MCAO sample, Figure 1). These venous systems of the brain drains into the confluence of the sinuses that separates and empties into the right and left internal jugular veins before returning to the heart (Meder et al., 1994). The majority of the venous blood samples were drawn on the ipsilateral side (affected MCAO side) of the animal. When the blood samples at the affected MCAO site were unable to be collected, the contralateral side (unaffected MCAO side) was used. It takes approximately 30 minutes to obtain the pre-pMCAO blood sample given the details of the MCAO procedure and anesthesia induction (represented by the shaded box for the variable time points, Figure 1). The venous blood sample was analyzed using iSTAT Portable Clinical Analyzer (Abbott Laboratories, Abbott Park, IL). Post-MCAO (approximately taken within seven minutes of occlusion), rats remained anesthetized on the operating table and venousblood sample was again collected (representing the post-MCAO sample) and analyzed with the iSTAT.

The animals that survived to 72 hours underwent MRI for brain imaging and prior to euthanization, the third venous blood sample was collected from the right atrium and analyzed with the iSTAT (Figure 1). Before we perfuse our animals with saline, they are opened and the third blood sample is taken from the right atrium. The right atrium provides a venous blood sample that is convenient to obtain (we are working in a time window and to expose the IJ would exceed this) and it is downstream from the IJ. Acid/base and electrolyte parameters collected included: pH, carbon dioxide (pCO_2), oxygen (pO_2), bicarbonate (HCO_3^-), base excess of extracellular fluid (Beecf), glucose, hematocrit, hemoglobin, and serum electrolytes, including ionized calcium (iCa^{2+}), potassium (K^+), and sodium (Na^+).

Occasionally during iSTAT blood sample collection we received individual readings errors and these errors were excluded from statistical tests. Reasons for failure of blood gas analysis reading include iSTAT errors (air bubbles in the blood sample, insufficient blood drawn from the animal, micro blood clots (heparinized tubes are used but it still occurs), faulty blood cartridge, or we could not obtain blood pre-, post-, at 72 hours pMCAO from the animals (vessel collapsed, insufficient amount of blood drawn for the cartridge reading, and/or our surgeons are handling post-operative period in a timely fashion and we try to minimize time closing up the animal post-MCAO.

Magnetic Resonance Imaging

MRI images were acquired on a 7T Bruker Clinscan horizontal bore system (7.0T, 30cm, 300Hz) equipped with a triple-axis gradient system (630 mT/m and 6300 T/m/s) with a closed cycle. Diffusion Tensor Imaging (DTI) images were acquired coronally with a fat saturated, double refocused echo planar sequence: 0.297 × 0.297 × 0.7 mm^3, TR/TE 2200/34, 128 × 128 matrix, 3 av, 12 slices, four b=0 volumes, 256 directions with b=800, in 28:23 minutes (Figure 2a). T2 weighted images were acquired coronally with a fat saturated, turbo spin echo sequence: 0.125 × 0.125 × 0.4 mm^3 resolution, TR/TE 6000/27, 192 × 192 matrix, 44 slices, is 9:03 (Figure 2c).

Male rats were anesthetized with an average of 2.25% isoflurane in oxygen, while female rats were anesthetized with an average of 1.75% isoflurane in oxygen using an MRI compatible CWE Inc. equipment (Ardmore, PA). They were held in place on a Bruker scanning bed with a tooth bar, ear bars, and tape. Body temperature, heart rate, and respiratory rates were continuously monitored throughout the MRI scans (SA Instruments, Inc., Stony Brook, NY). The animals were maintained at 37°C with a water heating system built into the scanning bed. The scanning procedure took approximately 40 minutes per animal. Rats needed to survive to 72 hours for both T2 and DTI imaging, but there were several occasions we euthanized animals early (mid-MRI) due to gasping breathing and some animals passed during scans (i.e we were able to collect T2 imaging but not DTI imaging).

MRI Processing for Infarct Volume and Edema Volume

The DTI and T2 MR images were analyzed by a blinded neuroradiologist who identified infarct volume and edema volume. These volumes were counted and this number was normalized to the number of images counted to provide a per section count. The volume of brain parenchyma demonstrating restricted diffusion (infarct volume) visibly affected were calculated by manual segmentation using ITK-SNAP software (www.itksnap.org, version 3.6, Figure 2b) (Yushkevich et al., 2006). The volume of brain parenchyma visibly affected by cerebral edema (edema volume) were calculated in a similar fashion (Figure 2d). The data are given as absolute volume in cubic millimeters. The calculation was based on all slices from each MR sequence.

Statistical Analysis

Data analysis began with a descriptive examination of all variables, including frequency distribution, means, and standard deviations. The power analysis was completed using G*Power software (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007). Given a desired power of 0.8 and a significance level for between-group comparisons set at $p < 0.05$, the required sample size was calculated to be 14 animals (i.e., $n = 7$ per group). All variables in the study were transformed to meet assumptions of normality. The transformation procedures began with Shapiro-Wilks and for measures with $p < 0.05$, natural log with a constant of 10 added to measure. Data from the aged male and female rats were analyzed using SPSS, version 24 software (IBM, Armonk, NY).

A repeated-measures analysis of variance (ANOVA) was used to examine between-group differences of blood gas parameters of the sexes at three time points, pre-MCAO, post-MCAO, and at 72 hours-MCAO. Paired samples t-test were used to compare venous blood gas mean values between pre- and post-MCAO for the animals that died prior to 72 hours, in addition to an independent t-test to compare venous blood gas mean values between pre-MCAO animals that survived versus died prior to 72 hours. A multiple linear regression with backward variable entry was performed with magnitude of change data from aged male and female rats to determine whether venous acid/base and electrolyte changes predicted infarct volume and/or edema volume. The variables were entered into the regression model in one step. The adjusted R^2 was used to determine the variance explained in infarct volume and/or edema by each variable. An assessment of multicollinearity was performed by inspecting correlation coefficients and tolerance/variance inflation factor values. Inspecting the coefficients table and if tolerance values are less than 0.10 or VIF is above 10, collinearity is present and was not found in final model. The P-to-P plot was used to assess the changes in pH, iCa^{2+}, and Na^{+} data sets for normal distribution. A Kaplan-Meier curve using Mantel-Haenszel log-rank test analysis was performed to compare survival curves between aged male and female rats. A Cox regression curve was used to assess the relationship of event-free survival in aged male and female rats using predictors of acid/base and electrolyte concentration parameters. A p-value of 0.05 was set a priori to determine statistical significance.

Sample Size

The total sample size from the permanent MCAO model were 31. These animals are furthered categorized into those that survived to 72 hours (n = 16; males = 7 and females = 9), and those that died prior to 72 hours (n = 15; males = 8 and females = 7).

RESULTS

Acid/Base and Electrolyte Values for Survival

Venous Blood Gas Differences Between Aged Male and Female Rats—A repeated-measures analysis of variance (ANOVA) was used to examine between-group differences of blood gas parameters of the sexes at three time points, pre-pMCAO, post-MCAO, and at 72 hours-MCAO (Table 1). Mauchly's test indicated that the assumption of sphericity had been violated for each blood gas parameter therefore degrees of freedom were corrected with Greenhouse-Geisser estimates of sphericity (Table 2). There was not a significant interaction effect between the three time points of blood gas parameters and sex (Table 3).

Acid/Base and Electrolyte Values for Death

We did not see any baseline differences between males and females for those rats who died prior to 72 hours, so we combined male and female animals together. There were significant differences in blood gas analysis seen from pre- to post-MCAO in animals that died prior to 72 hours (n = 15, Table 4). Among animals that died, decreases in pH (p = 0.026) and Na^{+} (0.039) were observed from pre- to post-MCAO. Increases were seen in blood values pre- to post-MCAO for pCO_2 (p = 0.015), HCO^{3-} (p = 0.001) and glucose (p = 0.014). There

were no significant differences seen in baseline (pre-MCAO) values from those animals that survived and those that died prior to 72 hours (Supplemental Table 5).

Predictors for Infarct Volume and Edema Volume

Blood gas and electrolyte changes from male and female rats were combined together for multiple linear regression analyses. Multiple regression analysis (n = 15) showed that the changes in pH from pre- to post-MCAO and in pH from post to 72 hours-MCAO were predictors for infarct volume ($F(2,14) = 14.77$, $p = 0.030$). These variables explained 81% of the total variance in the model, as predictors of infarct volume (Table 6).

Multiple regression analysis (n = 16) revealed that the changes in iCa^{2+} from post to 72 hours-MCAO and Na^{+} post to 72 hours-MCAO were predictors for edema volume ($F(2,15) = 12.32$, $p = 0.015$). These variables explained 75% of the total variance in the model, as predictors of edema volume (Table 7). The mean infarct size is 203.64 ± 61.41, while the mean edema volume is 124.04 ± 41.39. There is no significant difference ($p = 0.524$) in infarct size between aged male (n = 6) and female rats (n = 9) or in edema volume ($p = 0.730$) between aged male (n = 7) and female rats (n = 9).

Relationship Between MCAO and Mortality

A Kaplan-Meier curve with Mantel-Haenszel log-rank test was used to analyze event-free survival between aged male and female rats. There was no difference between male and female rats based on mortality ($p = 0.321$, data not shown). While no significant results were seen between males and females based on mortality, a Cox regression modeling was used to assess the relationship of event-free survival in aged male and female rats using predictors of acid/base and electrolyte parameters. There is a 3.25 times (or 325%) greater risk for mortality based on change in HCO^{3-} (pre- to post-MCAO), after controlling for change in Na^{+} and sex ($p = 0.025$). Aged rats within −2.00 to −7.00 cut-off range for HCO^{3-} experienced an increased risk for mortality (Figure 3).

DISCUSSION

Previously, we have reported that changes in venous blood gas parameters of pH and ionized calcium (pre- to post-MCAO) predicts infarct volume in young male rats (Martha et al., 2018). We now extend our findings to aged male and female rats after pMCAO. The venous blood gas profile after pMCAO significantly differs between the young and aged. Our aged rats (18 months equivalent to approximately 55–65 years old in humans (Quinn, 2005)) are a better age match for human stroke patients. A major issue in stroke research is the lack of translating findings from the rodent to the human patient. The almost uniform use of young male rats in stroke research is one of the contributing factors for this problem. Our expectations are that data acquired using aged rats should be more successful in translation to the human condition. While we did not see any differences in the venous blood between the sexes, we are still using both sexes in order to match the human population. Gender-specific responses to stroke therapies are well-documented, and most recently exemplified in the experience with the clinical trial showing that uric acid is effective with females but not male stroke patients (Llull et al., 2015).

Carbon dioxide and water is catalyzed by carbonic anhydrase, which results in the generation of bicarbonate and H^+ (Hamm, Nakhoul, & Hering-Smith, 2015). The H^+ proton facilitates transport of Na^+ across membranes. Other electrolytes and water follow the transport of Na^+ during ischemia, causing malignant cerebral edema. Bicarbonate production is low in blood under alkaline conditions, and is high in acidic conditions found in ischemia. Its purpose is to regulate pH homeostasis, and to facilitate anion/water transport. In heart ischemia reperfusion, the presence of bicarbonate causes increased oxidative damage (Queliconi et al., 2013), which leads to increased inflammation. While ELVO causes significant injury to the affected brain, the associated malignant edema injures surrounding otherwise unaffected brain, caused herniation and death (Dostovic, Dostovic, Smajlovic, Ibrahimagic, & Avdic, 2016). These early changes in bicarbonate predict mortality of aged rats of both sexes and provides a potential biomarker to be explored in human stroke patients.

The pH is tightly regulated in the blood and other tissues to maintain homeostasis. The accumulation of lactic acid and CO_2 in ischemic brain disrupts the normal pH balance (Back et al., 2000). Our study showed significant changes of pH as a predictor of infarct volume, but interestingly, a predictor that varies depending on the time point at which pH is measured. At the first timepoint (from pre- to post- MCAO), our results demonstrate that as changes in pH increased, infarct volume increased. At the second timepoint (from post-MCAO to 72 hours), as changes in pH increased, infarct volume decreased. These changes in pH taken together are predictors that affect infarct volume. The importance of this finding occurring in the post-MCAO to 72-hour mark is important for translational studies, because this period would be the most applicable as a prognostic tool for the human patient.

In addition to finding predictors related to infarct volume, we have also identified predictors related to edema, which can be a lethal consequence of cerebral infarction. Our results indicate that changes in ionized calcium and sodium post-MCAO to 72 hours were found to be predictors for edema volume. We found an inverse relationship with changes in ionized calcium and sodium (post-MCAO to 72 hours) to edema volume, as ionized calcium and sodium increases, edema volume decreases. To our knowledge neither ionized calcium, nor sodium are explored in the literature on edema volume as a stroke outcome. They are, however, discussed in connection with infarct volume, patient functional outcomes, and mortality. In human stroke patients, severe clinical presentation was seen after stroke onset, as was worsening of functional outcomes, and the potential for hemorrhagic conversion post-thrombectomy; these were associated with lower total serum calcium concentrations in peripheral venous blood (Guo et al., 2015; Guven, Cilliler, Koker, Sarikaya, & Comoglu, 2011; Ishfaq, Ullah, Akbar, Rahim, & Afridi, 2017). Conversely, better functional outcomes and decreased infarcts were associated with higher venous total serum calcium levels (Buck et al., 2007; Ovbiagele et al., 2006; Ovbiagele et al., 2008). These findings link positive stroke outcomes to increased serum calcium levels. Yet, also seen in human stroke patients were inconsistent results of associations between serum Na^+ levels and stroke severity. Patients with increased stroke severity and mortality exhibited associations with hyponatremia or lower Na^+ levels (Rodrigues, Staff, Fortunato, & McCullough, 2014; Soiza et al., 2015). While others found associations between increase stroke incidence and neurological worsening with higher venous serum Na^+ concentrations (Christensen &

Boysen, 2002; Farahmand, Choobi Anzali, Heshmat, Ghafouri, & Hamedanchi, 2013; Fofi et al., 2012). However, these studies relied on univariate analyses so multivariate analyses appear to add more understanding of the associations of biomarkers with functional or neurological outcomes.

Changes in bicarbonate levels were also found to be important predictor for increased risk for mortality. Our results from the Cox regression model indicated there is a 3.28 times greater risk for mortality based on magnitude of change in bicarbonate levels (range −2.00 to - 7.00) from pre- to post-MCAO. Bicarbonate is important for pH, electrolyte, and water balance, which is disturbed during ischemic stroke. Bicarbonate levels change rapidly after inserting the embolus into the internal carotid artery to block the middle cerebral artery, approximately within 7 minutes. Our data shows that the physiological buffering response of the rats is impaired post- pMCAO; they are unable to adjust their blood homeostasis, leading to mortality. To our knowledge, this is the earliest change in bicarbonate measurement after experimental stroke to be reported. Moreover, this finding shows that there are two rat subpopulations in which one is able to regulate bicarbonate and survive. The other group cannot regulate bicarbonate and the levels are significantly enhanced, which increases probability of death by 328%. If translated to humans, this would show that early bicarbonate levels would dictate treatment for the patient after stroke.

There is, however, some distinction between the pMCAO model in young and aged rats in acid/base balance and electrolyte concentrations as predictors of infarct volume. In our previous paper, we discovered as changes in pH and ionized calcium (from pre- to post-MCAO) increased, infarct volume decreased in young male rats (Martha et al., 2018). This finding is inverse from what we posit in our current results related to aged rats, which show that as changes in pH (from pre- to post-MCAO) increase, infarct volume increases. We also previously found that as changes in ionized calcium (from pre- to post- MCAO) increased, infarct volume decreased. These changes were not found in aged rats, rather there was an association with changes in ionized calcium at a different time point (post-MCAO to 72 hours) with an increase in edema volume. The differences between young and aged rats demonstrate that some blood gas and electrolyte parameters are age dependent, while others are not. Translation of findings from rodent to human has been problematic in stroke research with the almost exclusive use of young male rats. As demonstrated by this study, differences in the response to stroke arise between young and aged rats. These aged rats are a closer age match to human stroke patients and could be a better model for translation to humans.

The physiological significant of this study is that the response to ischemia is rapid within minutes. Water movement is associated with transport of electrolytes, primarily with sodium. Our data indicates that if the brain is able to maintain the electrolytes, ionized calcium and sodium, within the blood then edema is reduced. This study indicates that if buffering in the blood is unable to adjust their blood homeostasis then infarct volume and death will occur. This maintenance of pH is essential as acidosis will result in neural cell death to expand the infarct volume (Sherwood, Lee, Gormley, & Askwith, 2011; Ying, Han, Miller, & Swanson, 1999; Yushkevich et al., 2006). Bicarbonate, as discussed above, plays a

critical role in maintaining blood homeostasis and the dysregulation of bicarbonate. In fact, serum bicarbonate is associated with heart failure and mortality (Kendrick et al., 2017).

Studies investigating the effects of acid base balance in anesthesia are scarce, and to our knowledge, this is the first study analyzing blood gas parameters in aged rats under anesthesia. Others have evaluated young female rats that were subjected to three different types of anesthesia (pentobarbital, ketamine/xylazine, or zoletil) and after 20 minutes of administration, acid base balance and electrolyte concentrations were examined. All three groups blood samples were in a state of acidosis and hypoxia. Under pentobarbital the young rats experienced hypercapnia with elevated bicarbonate levels. While the rats exposed to ketamine/xylazine ranged from normocapnia to hypercapnia and a wide range of bicarbonate values, and the rats under zoletil were hypercapnic and normal levels of bicarbonate (Svorc, 2018). The pentobarbital findings are in congruence with our results. We also noticed the longer our animals were under isoflurane (at 72 hours during MRI procedure) they experienced severe levels of hypoxia or pO_2.

In conclusion, this study shows that blood chemistry in the systemic circulation responds rapidly to an ischemic event in the brain. We demonstrate the importance of identifying early significant changes in blood gases and electrolyte concentrations after induction of the permanent middle cerebral artery occlusion stroke, particularly as it relates to infarct volume, edema volume, and mortality. We found pH (pre- to post-MCAO and post-MCAO to 72 hours) was predictive of infarct volume; changes in ionized calcium and sodium (post-MCAO to 72 hours) were predictive of edema volume; and the changes of bicarbonate within the range of - 2.00 to −7.00 increased the risk for mortality. Of the three time points used, the post-MCAO and 72-hour time points would be most applicable to the human stroke patients since they receive treatment hours after having a stroke. Finally, bicarbonate stands out as the most promising indicator from our study, providing us with a predictive range for mortality but must be examined in the context of the human patient. Overall, these studies will provide acid/base and electrolyte concentrations as potential predictive biomarkers for stroke outcomes. These studies need be verified in human patients an at appropriate time points in patient care. However, the use of multivariate analyses appears to be more appropriate approach in determining predictive biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SIGNIFICANCE STATEMENT:

By evaluating acid/base balance and electrolyte levels, we can identify prognostic indicators that assist in predicting the severity of stroke outcomes. Cerebral infarction produces edema and electrolyte imbalances, factors that contribute to brain swelling, dysfunction, and mortality in stroke patients. Our findings reveal that acute changes in acid/base balance and electrolytes occur in aged rat model of stroke pMCAO. Changes in pH, ionized calcium, and sodium were predictive of infarct and edema volumes, while changes in bicarbonate indicated an increased risk of mortality. Further studies should examine if the interplay of acid/base and electrolyte levels relates to the pathophysiology in the human stroke condition.

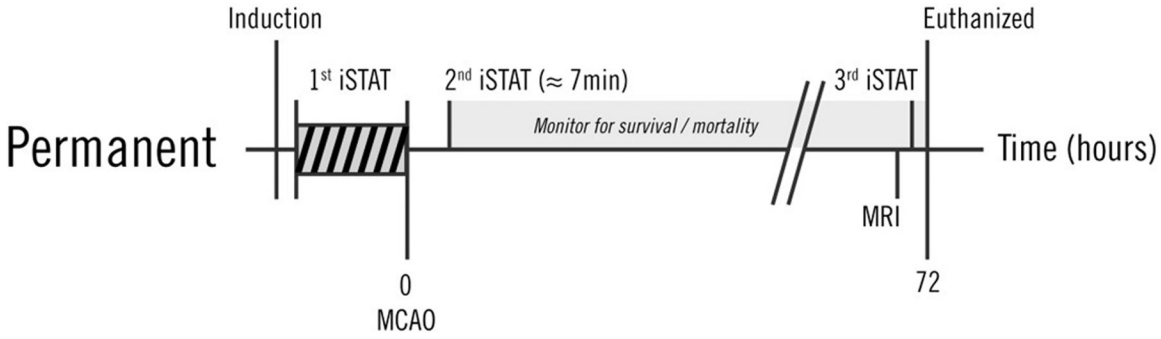

Figure 1:

Permanent Timeline

The first venous blood sample collection (represented by 1st iSTAT) to MCAO takes approximately 30 minutes (represented by the shaded box). The post-MCAO sample was taken approximately seven minutes after induction of the MCAO (represented by 2nd iSTAT). The MRI scans and 3rd iSTAT collection were obtained before euthanization at 72 hours.

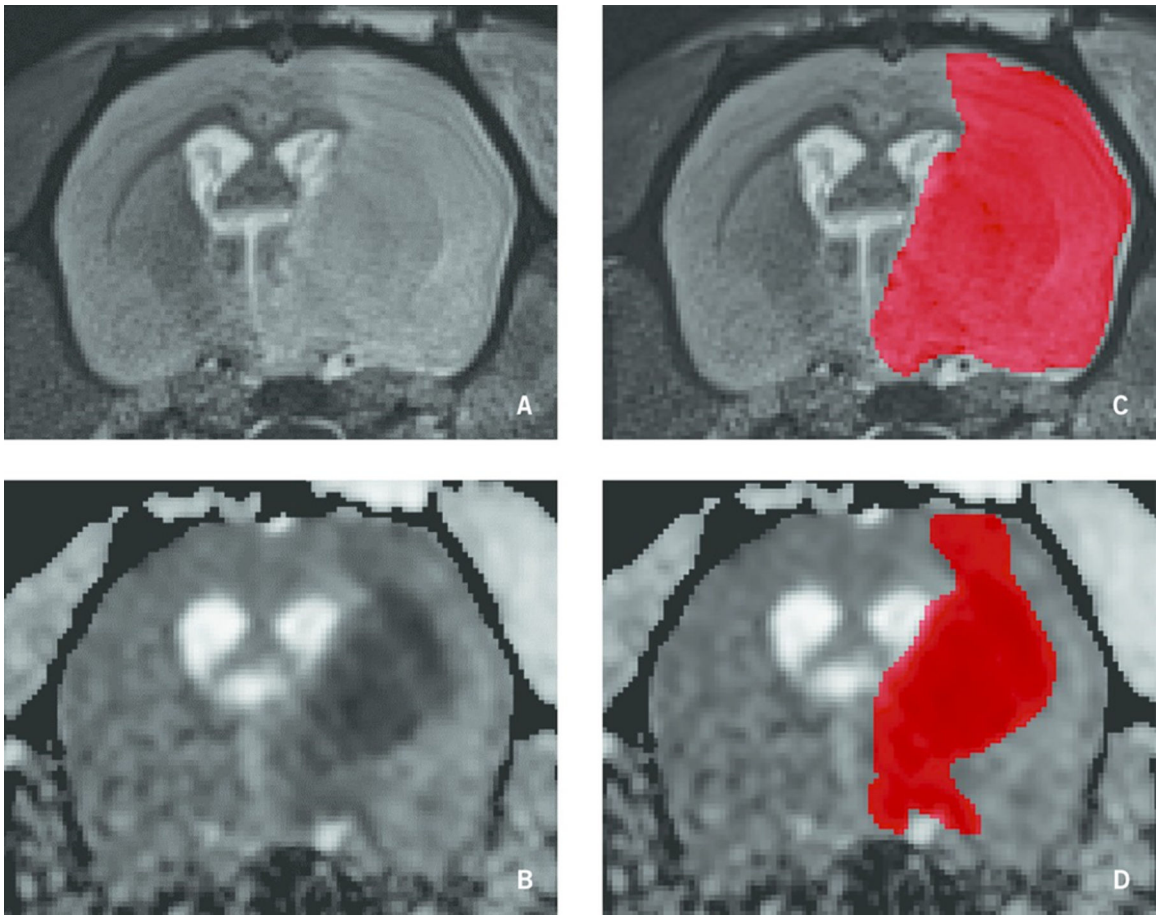

Figure 2.

A-D: MRI Images of Infarct and Edema Volumes

2a: Representative permanent-MCAO Diffusion Tensor Imaging (DTI) image of infarct volume.

2b: Representative permanent-MCAO T2-weighted image of edema volume.

2c: DTI imaging revealed the volume of brain parenchyma demonstrating restricted diffusion of infarct volume visibly affected after pMCAO. Shading indicates the calculation made by manual segmentation.

2d: T2 weighted imaging revealed the volume of brain parenchyma demonstrating restricted diffusion of edema volume visibly affected after pMCAO. Shading indicates the calculation made by manual segmentation.

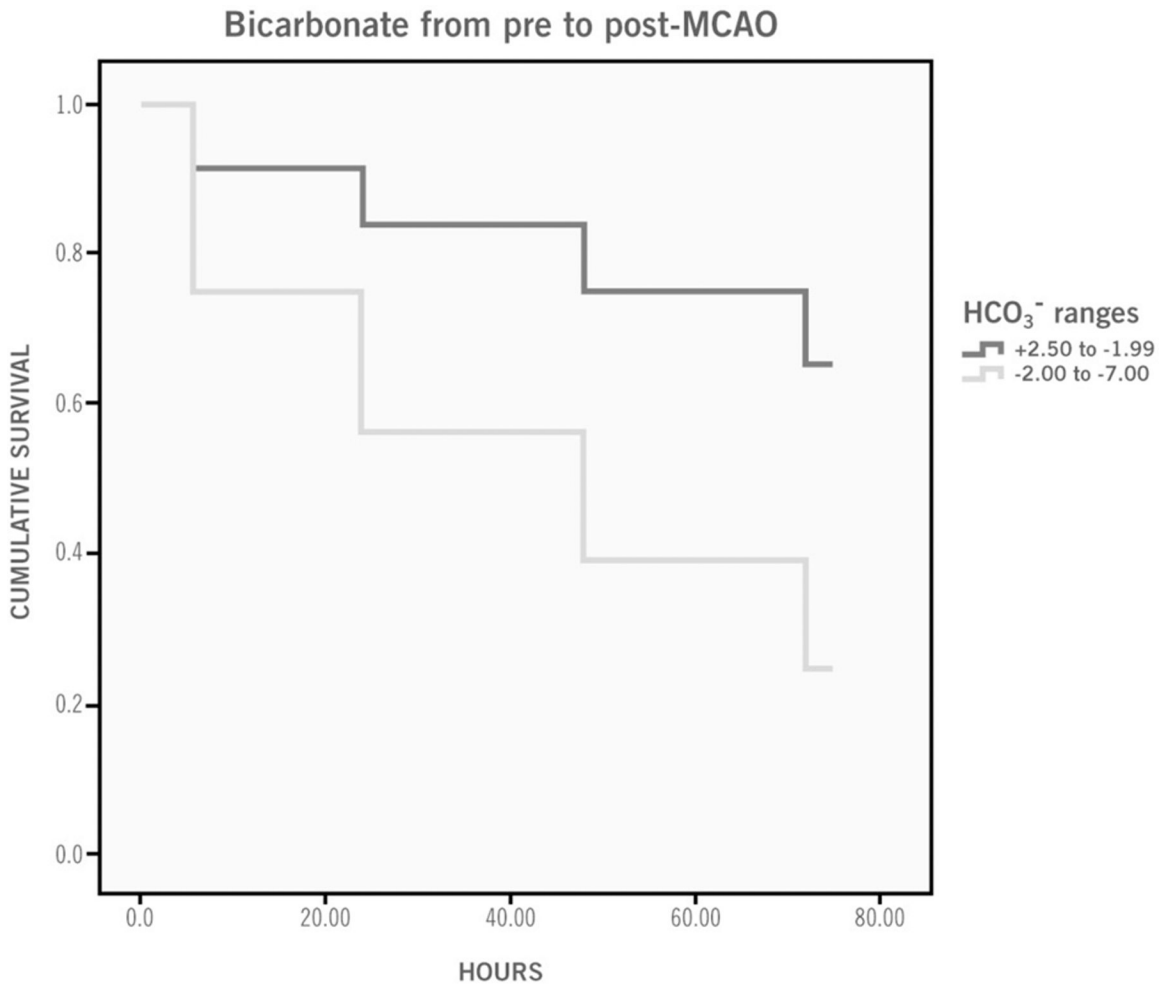

Figure 3:

Cox Regression and Mortality

Cox regression model analysis revealed a 3.25 times greater risk for mortality based on the magnitude of change in bicarbonate levels (range −2.00 to −7.00) from pre- to post-MCAO.

Table 1:

Repeated Measures ANOVA results of sex and blood gas parameters at three time points.

Parameters	All Rats Mean ± SD	Male Mean ± SD	Female Mean ± SD	P-value
pH				
pre-MCAO	7.30 ± 0.04	7.32 ± 0.04	7.29 ± 0.03	0.125
post-MCAO	7.23 ± 0.07	7.27 ± 0.04	7.20 ± 0.06	
72 hours	7.24 ± 0.04	7.26 ± 0.03	7.23 ± 0.04	
pCO_2 (mmHg)				
pre-MCAO	60.11 ± 6.29	58.38 ± 6.58	61.27 ± 7.23	0.213
post-MCAO	77.08 ± 6.77	71.05 ± 6.94	81.10 ± 6.06	
72 hours	74.68 ± 7.62	73.83 ± 7.66	75.24 ± 7.58	
pO_2 (mmHg)				
pre-MCAO	125.33 ± 13.87	127.00 ± 11.24	123.22 ± 15.56	0.409
post-MCAO	103.33 ± 14.72	102.50 ± 16.67	103.89 ± 12.21	
72 hours	9.93 ± 1.76	9.17 ± 1.48	10.44 ± 1.96	
Beecf (mmol/L)				
pre-MCAO	3.33 ± 0.49	4.50 ± 0.64	3.56 ± 0.35	0.612
post-MCAO	4.80 ± 0.27	5.33 ± 0.36	3.78 ± 0.20	
72 hours	5.00 ± 0.62	6.83 ± 0.81	3.76 ± 0.45	
HCO^{3-} (mmol/L)				
pre-MCAO	29.95 ± 1.59	30.35 ± 1.26	29.69 ± 1.83	0.317
post-MCAO	31.65 ± 2.03	32.27 ± 1.43	31.23 ± 2.34	
72 hours	32.47 ± 2.84	34.18 ± 2.88	31.33 ± 2.80	
Na^+ (mmol/L)				
pre-MCAO	137.60 ± 1.51	137.17 ± 1.72	137.89 ± 1.36	0.774
post-MCAO	136.00 ± 1.56	135.67 ± 1.37	137.22 ± 1.72	
72 hours	144.20 ± 3.35	143.33 ± 3.23	144.78 ± 3.58	
K^+ (mmol/L)				
pre-MCAO	3.73 ± 0.34	3.97 ± 0.21	3.57 ± 0.32	0.871
post-MCAO	3.87 ± 0.30	4.03 ± 0.31	3.77 ± 0.27	
72 hours	4.59 ± 0.48	4.77 ± 0.66	4.45 ± 0.30	
iCa^{2+} (mg/dL)				
pre-MCAO	1.01 ± 0.16	1.01 ± 0.11	1.02 ± 0.19	0.673
post-MCAO	1.03 ± 0.13	1.07 ± 0.15	1.01 ± 0.12	
72 hours	1.22 ± 0.13	1.27 ± 0.10	1.18 ± 0.14	
Glu (mg/dL)				
pre-MCAO	223.67 ± 22.67	237.67 ± 19.52	214.33 ± 24.81	0.712
post-MCAO	346.87 ± 36.26	357.67 ± 33.87	339.67 ± 38.36	
72 hours	156.67 ± 16.19	158.50 ± 18.63	155.44 ± 14.89	

Parameters	All Rats Mean ± SD	Male Mean ± SD	Female Mean ± SD	P-value
Hct (% PCV)				
pre-MCAO	40.13 ± 2.13	42.33 ± 2.07	38.67 ± 2.24	0.268
post-MCAO	41.23 ± 2.65	42.67 ± 2.80	39.45 ± 2.42	
72 hours	35.27 ± 1.20	37.17 ± 1.31	33.44 ± 1.13	
Hbg (g/dL)				
pre-MCAO	13.65 ± 0.76	14.40 ± 0.71	13.14 ± 0.77	0.158
post-MCAO	13.65 ± 0.68	14.53 ± 0.57	13.07 ± 0.72	
72 hours	11.99 ± 1.48	13.63 ± 1.20	10.90 ± 1.63	

Data are presented as mean ± standard deviations. Total sample size N = 16; n = 7 male rats, n = 9 female rats. P values represent the interaction of 3 time points x gender. Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Table 2:

Mauchly's test indicated that the assumption of sphericity had been violated for each blood gas parameter therefore degrees of freedom were corrected with Greenhouse-Geisser estimates of sphericity.

Variable	Approximate Chi-Square	*df*	Greenhouse-Geisser Estimate of Sphericity	Significance
pH	22.26	2	ε = 0.678	p <0.001
pCO_2	23.49	2	ε = 0.791	p <0.001
pO_2	20.45	2	ε = 0.550	p <0.001
HCO^{3-}	15.61	2	ε = 0.579	p <0.001
Beecf	17.75	2	ε = 0.564	p <0.001
Na^+	13.64	2	ε = 0.596	p <0.001
K^+	11.05	2	ε = 0.517	p <0.001
iCa^{2+}	12.26	2	ε = 0.502	p <0.001
Glu	8.31	2	ε = 0.667	p = 0.016
Hct	15.98	2	ε = 0.569	p <0.001
Hbg	17.06	2	ε = 0.572	p <0.001

Table 3:

There was not a significant interaction effect between the three time points of blood gas parameters and sex.

Variable	F	*df*	Significance
pH	2.27	2	p = 0.125
pCO_2	1.64	2	p = 0.213
pO_2	1.17	2	p =0.409
HCO^{3-}	1.12	2	p = 0.317
Beecf	0.31	2	p = 0.612
Na^+	0.12	2	p = 0.774
K^+	0.14	2	p = 0.871
iCa^{2+}	0.39	2	p = 0.673
Glu	0.22	2	p = 0.712
Hct	1.75	2	p = 0.268
Hbg	1.39	2	p = 0.158

Table 4:

Acid/base and electrolyte values for animals who died prior to 72 hours, time points include pre- and post-MCAO.

		Died Prior to 72 hours		
Parameters	***n***	**Pre-MCAO Mean ± SD**	**Post-MCAO Mean ± SD**	**P-value**
pH	15	7.31 ± 0.05	7.19 ± 0.12	**0.026**
pCO_2 (mmHg)	15	58.54 ± 4.80	91.13 ± 6.09	**0.015**
pO_2 (mmHg)	15	121.29 ± 14.41	119.57 ± 13.72	0.686
Beecf (mmol/L)	15	3.50 ± 0.52	3.52 ± 0.55	0.919
HCO^{3-} (mmol/L)	15	29.50 ± 2.24	35.67 ± 2.36	**0.001**
Na^+ (mmol/L)	15	137.19 ± 1.60	134.00 ± 1.67	**0.039**
K^+ (mmol/L)	15	3.81 ± 0.47	4.11 ± 0.43	0.151
iCa^{2+} (mg/dL)	15	1.02 ± 0.11	1.04 ± 0.13	0.657
Glu (mg/dL)	15	259.86 ± 31.99	360.00 ± 34.04	**0.014**
Hct (% PCV)	15	41.84 ± 2.48	42.86 ± 3.19	0.522
Hbg (g/dL)	15	13.99 ± 0.83	13.90 ± 1.10	0.589

Table 6:

Multiple linear regression variables predicting infarct volume in aged rats (n = 15)

Variable	β	P-value
ΔpH (pre- to post-MCAO)	0.667	0.031
ΔpH (post- to 72 hours-MCAO	−0.683	0.034

R^2 = 0.862, adjusted R^2 = 0.813, *df* = 2, F = 14.77, p = 0.030

Durbin-Watson = 1.35

Table 7:

Multiple linear regression variables predicting edema volume in aged rats (n = 16)

Variable	β	P-value
ΔNa^{+} (post- to 72 hours-MCAO)	−1.355	0.011
ΔiCa^{2+} (post- to 72 hours-MCAO)	−0.973	0.018

R^2 = 0.813, adjusted R^2 = 0.745, *df* = 2, F = 12.32, p = 0.015

Durbin-Watson = 2.13