Translational Evaluation of Acid/Base and Electrolyte Alterations in Rodent Model of Focal Ischemia

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Background and Purpose: Acid/base and electrolytes could provide clinically valuable information about cerebral infarct core and penumbra. We evaluated associations between acid/base and electrolyte changes and outcomes in 2 rat models of stroke, permanent, and transient middle cerebral artery occlusion. *Methods:* Three-month old Sprague-Dawley rats underwent permanent or transient middle cerebral artery occlusion. Pre- and post-middle cerebral artery occlusion venous samples for permanent and transient models provided pH, carbon dioxide, oxygen, glucose, and electrolyte values of ionized calcium, potassium, and sodium. Multiple regression determined predictors of infarct volume from these values, and Kaplan-Meier curve analyzed morality between permanent and transient middle cerebral artery occlusion models. *Results:* Analysis indicated significant differences in the blood gas and electrolytes between pre- to post-middle cerebral artery occlusion. A decrease in pH and sodium with increases in carbon dioxide, potassium, ionized calcium, and glucose changes were found in both middle cerebral artery occlusion models; while hematocrit and hemoglobin were significant in the transient model. pH and ionized calcium were predictors of infarct volume in the permanent model, as changes in pH and ionized calcium decreased, infarct volume increased. *Conclusions:* There are acute changes in acid/base balance and electrolytes during stroke in transient and permanent rodent models. Additionally, we found pH and ionized calcium changes predicted stroke volume in the permanent middle cerebral artery occlusion model. These preliminary findings are novel, and warrant further exploration in human conditions.

Key words: Focal ischemia—acid/base balance—electrolytes—early blood chemistry changes—infarct volume

Introduction

Approximately 87% of strokes are ischemic, and are characterized by blockage of blood flow in the brain from a thrombus or embolus.[1] Neurological insult occurs immediately when the cerebral artery is occluded, as neural tissue is deprived of oxygenated blood, glucose, and other nutrients.[2] The penumbra, the area surrounding the infarct, also includes tissue at risk of cell death, and will

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expand to adjacent areas if reperfusion does not occur.[2] When there are instabilities in cerebral blood flow (CBF), disturbances in brain metabolism occur, causing shifts in water and ion concentrations. With decreasing CBF, the blood downstream of the occlusion undergoes biochemical changes. Disruption in blood flow reduces oxygen (O_2) and glucose, and leads adenosine triphosphate to be mismatched with use and production.[3] Cerebral ischemia occurs when cerebral O_2 supply fails to meet cerebral metabolic demand. Reduction in CBF causes lactic acid and carbon dioxide (CO_2) accumulation.[4-6] Furthermore, electrolyte concentrations such as sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) are sensitive to pH changes to maintain cellular structure and function.[3] Dysregulation is additive, as disruption of the Na^+/Ca^{2+} pump leads to an increased concentration of intracellular Ca^{2+}.[7] Increased calcium can trigger apoptosis, depolarization that activates lipases to break down neuron cell membranes, and mitochondrial dysfunction, leading to the generation of free radicals and reactive oxygen species.[7]

Several studies have investigated the relationship of venous and arterial blood gas parameters in critically ill human patients,[8-13] and in rodent models[14,15] demonstrating correlations between the blood gas values. Knowledge of blood gases could provide clinically valuable information about the cerebral infarct core and penumbra. To date, few studies have evaluated acid/base balance and electrolyte changes occurring within a few minutes of focal ischemia and occlusion. The aim of our study was to evaluate these changes, and to correlate them with infarct volume and/or mortality in 2 different rat models of stroke: permanent middle cerebral artery occlusion (MCAO) and transient MCAO. The permanent-MCAO model would mimic the natural history of large vessel occlusive stroke, while the transient-MCAO model accounts for recanalization seen in current treatment states that aim to re-establish flow.

Materials and Methods

Permanent and Transient MCAO Model

Three-month old Sprague-Dawley rats (ENVIGO, Indianapolis, IN) were used for all procedures. The rats weighed between 300 and 350 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 through our Institutional Animal Care and Use Committee. Animals were kept in a climate controlled room on a 12-hour light and dark cycle (0700-1900) with free access to food and water. For both models, animals were administered vehicle of phosphate buffered saline (PBS) at 7.4, at 6, 24, and 48 hours after MCAO. This work was supported by National Institutes of Health, National Institute of Neurological Disorders and Stroke (NINDS), grant number R01 NS091146.

For the permanent middle cerebral artery occlusion (pMCAO) procedure, as previously described,[16] animals were placed in an induction chamber and anesthetized with 5% isoflurane/oxygen. A constant flow of 3%-4% isoflurane in 100% oxygen at a rate of 1 L/min was maintained during the procedure. A vertical incision was made near the sternum to expose the right common carotid artery, and then a dissection was performed to isolate the common carotid and its branches. The first clamp was placed on the internal carotid prior to the pterygopalatine/internal cerebral artery bifurcation, while the second clamp was placed on the common carotid artery. The placement of the second clamp further in the posterior direction allows more room for maneuvering during filament insertion. The external carotid was isolated, and used to access the arterial system. A 40 mm nylon monofilament was fed distally into the intracranial artery to approximately 25 mm, and then sutured in place to obtain permanent occlusion of the middle cerebral artery (MCA), M1 segment. The incision was closed with the filament in-place. A Laser Doppler (Moore Lab Instruments, Farmington, CT) monitored blood flow during the process. Animals that did not show 60% reduction in perfusion of blood flow after placement of the monofilament were excluded from the study. The transient MCAO (tMCAO) followed the same procedure, but the monofilament was removed and flow re-established after 60 minutes. The transient-MCAO rats (n = 19) were euthanized at 72 hours. The permanent-MCAO rats were euthanized at either 72 (n = 7) or 96 hours (n = 11). Brains from both models were harvested for infarct measurements.

Blood gas samples were collected and analyzed pre- and post-MCAO. An internal jugular line was inserted, prior to MCAO monofilament placement but after anesthesia induction, and an approximately .5 mL of venous blood sample was collected (representing the pre-MCAO sample). These venous systems of the brain drains into the sinus of confluence which splits to form the internal jugular veins, which is the final collecting port before returning to the heart.[17] 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 site were unable to be obtained, the blood draw occurred on the contralateral side (unaffected MCAO side). While the ipsilateral side location is preferred for consistency in our blood draw protocol, the blood is analogous due to the venous anatomy. It takes approximately 30 minutes to obtain the blood sample given the details of the MCAO procedure and anesthesia induction (represented by the shaded box for the variable time points, Figs 3a and 5a). The sample was analyzed using iSTAT Portable Clinical Analyzer (Abbott Laboratories, Abbott Park, IL). After pMCAO (approximately 7 minutes), rats remained anesthetized on the operating table and venous blood sample was again collected (representing the post-MCAO sample) and analyzed with the