Evidence of Apoptosis in a Region of Decreased $^{23}$Na MR Signal after Acute Focal Cerebral Ischemia

Robert BARTHA1, Mathew HOGAN2, Nagalingum RAJAKUMAR3, Sarah HENDERSON1, Ravi S MENON1
1The John P. Robarts Research Institute, London, ON Canada; 2Neuroscience Research Institute, University of Ottawa, Ottawa, ON Canada; 3Department of Physiology, University of Western Ontario, London, ON Canada;

Introduction
Focal cerebral ischemia results in severe oxygen deprivation (hypoxia) to a localized tissue region and graded deprivation to surrounding tissue (penumbra). Although hypoxic tissue dies in minutes, penumbral tissue can remain viable for hours but may eventually die by apoptosis. The clinical identification of viable tissue at risk in acute patients may justify aggressive stroke treatment using potentially harmful clot lysing or neuroprotective agents. MR diffusion and perfusion weighted imaging have been partially successful in the delineation of penumbral tissue, however $^{23}$Na MR imaging may be a more direct and sensitive marker (1). The purpose of this study was to determine whether the time course of total $^{23}$Na signal change could discriminate between penumbral and necrotic tissue during the first six hours of focal cerebral ischemia.

Methods
Three New Zealand white male rabbits were anesthetized with 1-1.5% isoflurane and then ventilated to normocapnia. Rectal temperature was maintained between 36.5-37.5 °C. Following initial surgical preparation, rabbits were transported to a Varian/Siemens Unity Inova 4.0 Tesla MR scanner and placed in a custom built holder. Baseline volumetric T1 (TR/TE = 15/8 ms), and T2 (FSE, TR/TE = 3000/3 ms) weighted anatomic images were acquired followed by baseline 3D gradient echo volumetric single quantum $^{23}$Na images (TR/TE = 20/5 ms, 8 slices, 5 mm thickness, FOV = 8 cm, readout bandwidth = 6.4 kHz, 20 minute acquisition) using a custom built $^{23}$Na TORO coil (2). To minimize echo time and therefore increase signal to noise ratio, $^{23}$Na images were acquired using a fractional (65%) k-space readout. After baseline imaging, the rabbit was withdrawn from the magnet and ischemia was initiated by injecting a 1.5 cm blood clot into the right internal carotid artery followed by a 5 cc saline bolus to noise ratio, $^{23}$Na images were acquired using a fractional (65%) k-space readout. After baseline imaging, the rabbit was withdrawn from the magnet and ischemia was initiated by injecting a 1.5 cm blood clot into the right internal carotid artery followed by a 5 cc saline bolus lodging it in the proximal portion of the middle cerebral artery (MCA). The rabbit was replaced in the magnet and sodium images were continuously acquired for six hours. Rabbits were then sacrificed and histology obtained. Initially the brains were cut into 5 mm slices for TTC staining and then prepared for immunocytochemical staining for cleaved caspase-3, an early marker of apoptosis. The time course of $^{23}$Na signal intensity was examined for each pixel using Stimulate (3). Pixels that were significantly correlated (p<0.01) with monotonically increasing and decreasing functions were identified. Regions showing abnormal $^{23}$Na signal evolution were then further examined by immunocytochemistry.

Results
Figure one shows a 5 mm T1 weighted transverse anatomical image (left) and corresponding total $^{23}$Na image (right) showing distinct regions of hyperintensity (arrows). More specifically, tissue regions exhibiting one of three distinct $^{23}$Na signal time courses were found in all three rabbits: no change, increasing intensity, and decreasing intensity. These distinct tissue regions were further characterized by histology. Necrotic tissue was identified in two of the three rabbits by TTC staining (Figure 3-left, tissue without viable mitochondria appears white in TTC stained sections) and corresponded to the regions that exhibited increased $^{23}$Na intensity (dashed outlined regions in Figure 1). Decreased $^{23}$Na intensity was observed in isolated regions surrounding the increased $^{23}$Na regions (solid outlined regions in Figure 1-left). Immunocytochemical staining of the regions with increased $^{23}$Na signal intensity was positive for cleaved caspase-3 in one rabbit only (bright dots in Figure 3-right) indicating that apoptosis had been initiated in this region. There was no evidence of apoptotic activity in necrotic regions or in tissue contralateral to the stroke in any rabbits.

Discussion
Necrotic tissue identified by TTC staining corresponded to the region of increased $^{23}$Na during the first six hours of focal cerebral ischemia in two rabbits (necrotic tissue was not observed in the third rabbit). This result is consistent with previous reports (1, 4) of increased $^{23}$Na signal following ischemia. In addition, distinct regions of decreased $^{23}$Na signal intensity were observed in all three rabbits, with evidence of apoptosis found in one such region in one rabbit. Since apoptosis can be initiated long after the initial infarct, this process may not have progressed to the point of detection by six hours in all animals. Future studies will determine whether decreased $^{23}$Na intensity preceeds and can predict future apoptotic activity, and whether regions of decreased $^{23}$Na represent potentially salvageable tissue that may be responsive to stroke therapies.

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