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MRI Quantitation of Edema in Focal Cerebral Ischemia in Cats: Correlation with Cytochrome aa_3 Oxidation State

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^{1}H MRI permits detection of edema in the brain. In a middle cerebral artery stroke model in the cat, we found a significant correlation between an edema index based on MRI and a sensitive metabolic index of ischemia, the *in vivo* oxidation status of mitochondrial cytochrome aa_3 determined by near-infrared reflectance spectrophotometry ($r = -0.70$, $\alpha = 0.001$). This result suggests that a simple, noninvasive study using MRI can provide an index of the extent of ischemic damage in an experimental acute stroke model.

^{1}H MRI is sensitive to the presence of edema in the brain. It would be of interest to know if MRI can provide a quantitative measure of the extent of brain damage following a stroke. If so, then MRI might provide a simple noninvasive means for examining objectively the course of events in chronic stroke models. A sensitive *in vivo* monitor of the metabolic status of the brain is provided by the redox state of cytochrome aa_3 measured by near-infrared reflectance spectrophotometry (*1–3*). In the hypoxic rat brain the redox state of cytochrome aa_3 correlated with decreased concentrations of phosphocreatine (PCr), which is the most labile of the high-energy phosphate stores (*4*). We chose a cat stroke model for our studies because the cat's cerebral pathophysiology following occlusion of the middle cerebral artery (MCA) is well documented in experimental stroke research (*5*). In the course of these studies, we found a significant correlation between an MRI edema index and the *in vivo* cytochrome aa_3 oxidation state.

Cats (3–5 kg) were anesthetized with ketamine and isoflurane and the left eye was enucleated. The left MCA was isolated transorbitally and looped with a suture accessible through the orbit. After recovery for 3–7 days, the animals were reanesthetized and catheters for blood sampling and infusion inserted into the right and left femoral artery and vein. An endotracheal tube was inserted to permit ventilatory manipulation. Probes for the near-infrared reflectance spectrophotometer were placed on the exposed, intact skull over the medial portion of the left central sulcus. Acute infarction of the left parietal motor area was induced by tightening the looped suture and simultaneously clamping both internal carotid arteries. After 2 h, the carotid clamps were released and the cat was ventilated with 50 or 100% O_2. Hemoglobin, oxyhemoglobin, and cytochrome aa_3, which is the terminal step in neuronal respiration, were

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monitored by transcranial near-infrared (NIR) reflectance spectrophotometry during and for 8 h post-stroke. At 9 h, T_1- and T_2-weighted MRI imaging was performed to outline the area of infarction and to obtain a numeric assessment of brain edema.

Of the 24 animals in the study, 9 were untreated post-stroke, 7 were isovolemically transfused with Dextran-40, and 8 were isovolemically transfused with Fluosol-DA 20% (Alpha Therapeutics, Los Angeles, CA). These pharmacologic manipulations were used at F_iO_2's of 0.5 in some animals and 1.0 in others. The combinations of pharmacologic manipulations and F_iO_2's served to generate a range of metabolic responses following the ischemic event.

Hemoglobin, oxyhemoglobin, and cytochrome aa_3 oxidation state were recorded *in vivo* by a multiwavelength, differential NIR spectrophotometer (OMNI 4R, International Instrument Laboratory, Inc., Durham, NC) with monochromator light sources at 775, 815, 872, and 904 nm. The optical probes at the end of the fiber-optic bundle were placed on the exposed, intact skull over the medial portion of the left central sulcus lateral to the longitudinal fissure. This includes the watershed area between the left MCA and the left anterior cerebral artery (ACA) and represents the ischemic penumbra following occlusion of the left MCA. Measurements were made in the reflectance mode. The optical probes for transmission and reception were placed at right angles to each other on the skull and shielded from ambient light with IR-resistant tape and layers of black cloth (low-intensity blue light was used in the laboratory during the experiment). Dark current signal from the photomultiplier tube was used as a reference to correct for background fluctuations. NIR signals were recorded before ligating the MCA and clamping the carotids to induce the stroke, and at the end of the 2-h period of carotid clamping. The latter signal is obtained at a time at which cytochrome aa_3 is largely in a reduced state and is assigned a relative value of -100%. At 1 h after carotid clamping was released the signal was recorded, and O_2 (50 or 100%) was started. Fifteen minutes later the NIR signal was again recorded and now reflects a relatively oxidized state of cytochrome aa_3. This was assigned a value of 0%. The range between this value and the value at the end of carotid clamping (-100%) established the relative signal scale, which we define as the total labile signal for our experiments. The signal was recorded until 8 h post-stroke. The 8-h signal is termed the cumulative aa_3 signal expressed as a percentage (positive or negative) change from the 0% value defined above. A positive value indicates a more oxidized state and a negative value indicates a more reduced state.

Changes in the hemoglobin and oxyhemoglobin signals were followed to provide information about the oxygenation state of the hemoglobin in the ischemic region. Ordinarily, their algebraic sum can be used to provide an index of the tissue blood volume. However, in the eight perfluorocarbon-treated cats and in the seven dextran-treated cats, in which blood was partially replaced in such a manner as to maintain constant vascular volume, the algebraic sum of the hemoglobin and oxyhemoglobin signals cannot be used to indicate tissue vascular volume. And, in the eight cats in which part of their blood was replaced with an O_2-carrying perfluorocarbon, the oxyhemoglobin signal cannot be used to indicate tissue oxygenation.

The cytochrome aa_3 Cu absorption band near 815 nm is overlapped by those of hemoglobin and oxyhemoglobin. Therefore, signals were collected at the four different wavelengths listed above, and corrections for overlapping hemoglobin and oxyhe-

moglobin signals were made by an algorithm derived experimentally using standard conditions by the manufacturer. The specific algorithm for our instrument, kindly provided to us by Franz Jobsis, Ph.D., Department of Physiology, Duke University, Durham, North Carolina, is given in Eq. [1], in which the coefficients are instrument-dependent but stable over time:

$$\Delta Abs_{aa3} = -1.032\Delta Abs_{775} + 5.139\Delta Abs_{815} + 1.475\Delta Abs_{872} - 5.237\Delta Abs_{904}. \quad [1]$$

Since the cytochrome aa_3 oxidation state may under many conditions be expected to track the oxygenation state of hemoglobin, it is important to validate the multi-wavelength, differential recording technique and the algorithm used to derive the aa_3 signal. This was accomplished in experiments in which changes in aa_3 occurred in the absence of hemoglobin (blood replaced by an O_2-carrying perfluorocarbon) (*1*) or in the absence of changes in oxyhemoglobin (*2, 3*). A similar approach was used to validate cytochrome aa_3 measurements using wavelengths in the visible spectrum (*6*).

At the ninth hour post-stroke, brains were imaged *in vivo* in a 1-T Siemens MR imager using a Helmholtz coil permitting a 15 × 15-cm field of view. Contiguous 4-mm axial T_1-weighted MRIs were obtained in 256 × 256 matrices with TR 0.550 s, TE 17 ms, two acquisitions. Axial T_2-weighted images were obtained in 256 × 256 matrices with TR 1.9 s, TE 90 ms, two acquisitions. An index of the extent of edema in each T_2-weighted MR image was obtained from the mean pixel intensities in 0.1-cm^2 areas in each of three positions (top, middle, and bottom of the parietal lobe) in each of three adjacent slices centered at the lateral ventricles. The mean pixel intensities on the left (ischemic) side were expressed as ratios of those on the right. An index of the degree of edema in T_1-weighted images was obtained by expressing the width of the left hemisphere as a ratio of that of the right hemisphere in each of the same three slices. A single quantitative index of edema (MRI edema index) was obtained by summing the 12 ratios above and multiplying by 100. In five normal cats, the mean signal ratios of the individual T_2-weighted images (three in each cat) were 1.002 ± 0.075 (SD), which shows that the RF field of the imaging coil is reasonably uniform through the regions of interest.

The ischemia produced by the method described was extensive, but none of the infarctions were hemorrhagic, as determined by gross histological examination. Typical post-ischemia T_1- and T_2-weighted images together with the edema measurement sites in one slice are presented in Fig. 1. The post-ischemia pharmacologic manipulations resulted in a wide range of MRI edema indices as well as a wide range of cumulative aa_3 oxidation states. In Fig. 2, the cumulative aa_3 oxidation state signal at 8 h for each of the 24 animals is plotted as a function of the corresponding MRI edema index at 9 h. First-order regression analysis shows that measurements of cerebral edema by MRI correlate linearly with changes in cytochrome aa_3 oxidation measured by NIR with a correlation coefficient, r, of −0.70, that has a significance, α, of 0.001.

The MRI edema index did not correlate with the cumulative hemoglobin signal ($r = 0.005$, $\alpha > 0.90$) or the oxyhemoglobin signal ($r = 0.176$, $\alpha > 0.30$), and there was only a weak correlation of the cytochrome aa_3 signal change with the oxyhemoglobin signal change ($r = 0.43$, $0.05 < \alpha < 0.02$). These results are not surprising in view

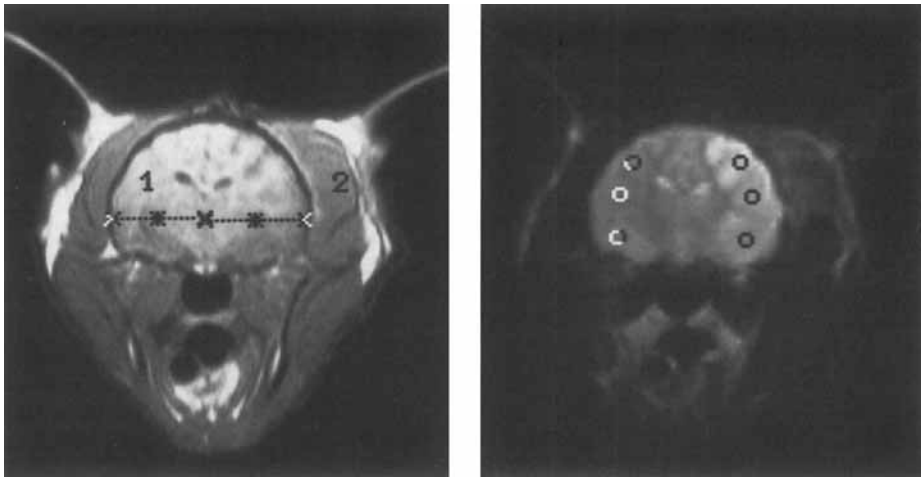

FIG. 1. T_1-weighted MR image (left) showing displacement of intercerebral fissure of cat brain at 9 h post-stroke. T_2-weighted MR image (right) of the same slice, showing edema in the left hemisphere. The small circles mark the sites of measurement of mean pixel intensity.

of our experimental design, which in the perfluorocarbon-treated animals causes a dissociation between O_2 delivery and hemoglobin concentration. The weak correlations between either the MRI edema index or the signal change of cytochrome aa_3 and the signal change of oxyhemoglobin, coupled with the strong correlation between the MRI edema index and the cytochrome aa_3 signal, suggest that the aa_3 signal is not simply tracking the oxyhemoglobin signal. This result helps to validate the technique used to extract the aa_3 signal in the presence of overlapping hemoglobin signals (*1–3*).

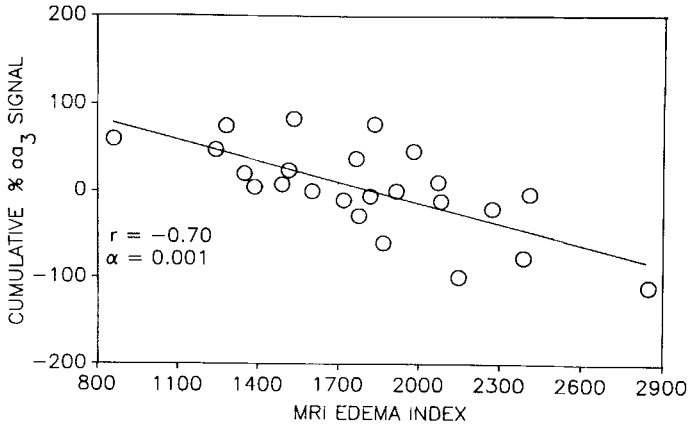

FIG. 2. Cumulative cytochrome aa_3 oxidation levels plotted as a function of MRI edema indices for 24 animals.

Our results complement those obtained by Miyake *et al.* (*7*) in a similar cat stroke model studied over 6 h. A progressive increase in the ^{23}Na NMR signal, which indicates increased edema, correlated with the rise in lactate measured by ^{1}H MRS. These changes were accompanied by a decrease in tissue blood volume measured by NIR spectrophotometry. Hilal *et al.* (*8*) imaged the increased sodium in the hemisphere of cat brains infarcted by middle carotid artery occlusion.

It is well known that metabolic inhibition sufficient to cause depletion of ATP leads to cell swelling (*9*). However, events following brain ischemia are complex; cellular changes occur which may affect the distribution of water within the cell, with swelling of intracellular organelles like endoplasmic reticulum and mitochondria, in the absence of changes in total tissue water (*10*). It is conceivable that redistribution of cellular water causes a longer T_1 and T_2 of some of the water, accounting in part for the intensity of the "edema index" in our study. It has not yet been determined in the cat stroke model if the oxidation status of cytochrome aa_3 correlates with the PCr or ATP concentrations. Whether the cytochrome aa_3 oxidation status or the MRI edema index correlates with histopathologic changes in cellular structure also remains to be determined. Nevertheless, our results suggest that ^{1}H MRI can provide an index of the extent of metabolic insult in the acute phase of experimental cerebral ischemia.

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