Regional Variations in the Apparent Diffusion Coefficient and the Intracellular Distribution of Water in Rat Brain During Acute Focal Ischemia

Kai-Feng Liu, MD; Fuhai Li, MD; Turgut Tatlisumak, MD; Julio H. Garcia, MD; Christopher H. Sotak, PhD; Marc Fisher, MD; Joseph D. Fenstermacher, PhD

Background and Purpose—The apparent diffusion coefficient of water (ADC) rapidly drops in ischemic tissue after cerebral artery occlusion. This acute drop is thought to be caused by the loss of extracellular fluid and the gain of intracellular fluid. To test the latter possibility, changes in ADC and the size of several cellular compartments were assessed in 3 regions of rat brain at the end of 90 minutes of focal cerebral ischemia.

Methods—One middle cerebral artery was permanently occluded in 8 Sprague-Dawley rats; sham occlusions were performed in 2 other rats. ADC maps were generated 90 minutes later, and the brains were immediately perfusion fixed. Three regions of interest (ROIs) were defined on the basis of ADC range. Various neuronal, astrocytic, and capillary compartments in each ROI were quantified with light and electron microscopy.

Results—At the end of 90 minutes of ischemia, mean ADC was normal in the cortex of sham-operated rats and the contralateral cortex of ischemic rats (ROI-a), 25% lower in the ipsilateral frontoparietal cortex (ROI-b), and 45% lower in the ischemic lateral caudoputamen (ROI-c). At this time, the frequency of swollen astrocytic cell bodies and volume of swollen dendrites and astrocytic processes in neuropil were ROI-a<ROI-b<ROI-c. In ROI-b and ROI-c, 40% and 60% of the neurons, respectively, were shrunken; the shrunken neurons were ~25% smaller in ROI-c than in ROI-b. In these areas, many capillary endothelial cells, pericytes, and perivascular foot processes were swollen.

Conclusions—The initial lowering of ADC during focal ischemia probably is the result of not only the acute loss of extracellular fluid and concomitant swelling of various cellular compartments but also concurrent neuronal shrinkage. (Stroke. 2001;32:1897-1905.)

Key Words: brain edema ■ cerebral ischemia ■ MRI ■ rats

Diffusion-weighted MRI (DWI) readily detects ischemic changes induced by cerebral arterial occlusion in the brains of both humans1–3 and animals.4–8 Immediately after occlusion of a cerebral artery, the ischemic regions appear hyperintense on DWI, which indicates a decline in the apparent diffusion coefficient of water (ADC).9 This acute drop in ADC has been ascribed to brain cell swelling (cytotoxic edema) with an accompanying decrease in the extracellular space, the result of ATP depletion, tissue acidosis, and failure of ion and volume regulation.10–16 In 2 of these studies, however, it was noted that the nucleus and perikaryon of some neurons shrank when ADC decreased during the acute phase of focal ischemia.10,16 Accordingly, the energy depletion and other metabolic disturbances caused by greatly reduced blood flow may not affect all brain compartments similarly, swelling some but shrinking others.

The present study was designed to determine whether the decline in ADC 90 minutes after unilateral middle cerebral artery (MCA) occlusion is correlated with not only acute swelling of various cellular compartments but also shrinkage of the neuronal soma. Three ranges of ADC values were used to select regions of interest (ROIs) for concurrent structural and ultrastructural analysis after 90 minutes of MCA occlusion. The quantified morphological parameters were the (1) frequency of normal and shrunken neuronal cell bodies, (2) neuronal size, (3) frequency of swollen astrocytes, (4) space of swollen dendrites and astrocytic processes in the neuropil, and (5) areas of perivascular foot processes, capillary endothelial cells, and capillary lumina.

Materials and Methods

Induction of Focal Brain Ischemia

All experiments were conducted according to the guidelines issued by our institutional animal care committee and were in compliance with regulations formulated by the US Department of Agriculture.17

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Sprague-Dawley rats weighing 300 to 350 g were anesthetized with an intraperitoneal injection of chloral hydrate (400 mg/kg). A PE-50 catheter was inserted into the left femoral artery to monitor arterial blood pressure and to obtain samples for blood gas measurements. Eight animals underwent 90 minutes of permanent MCA occlusion as originally described by Kozumi et al. Briefly, the right external carotid, the right occipital, and the right common carotid arteries were exposed and ligated proximally. A silicone-coated 4-0 nylon monofilament was inserted into the right common carotid artery through an arterial incision made 3 mm below the carotid bifurcation. The filament was advanced into the right internal carotid artery until mild resistance was felt (~17 mm above the bifurcation), indicating occlusion of the origin of the MCA. Two other animals underwent a sham operation in which the filament was advanced ~10 mm above the carotid bifurcation.

**DWI Measurements**

Immediately after surgery, the rats were placed in a homemade 1H birdcage imaging coil in which the animal’s head was held immobile with ear and tooth bars. In the MRI unit, anesthesia was maintained with 1% isoflurane (delivered in air at 1 L/min), and body temperature was continuously monitored by rectal probe and maintained at 37°C. A CSI-II 2.0-T/45-cm imaging spectrometer (General Electric Medical System) operating at 85.56 MHz for 1H and equipped with self-shielded gradient coils was used for this study. The diameter of the bore was 15 cm; the maximal deliverable gradient strength was ±20 G/cm. An 8-slice, diffusion-weighted, echo-planar image of 2-mm thickness was used to generate ADC maps. Half-sine-shaped diffusion gradients were used along the z axis (from the head to the tail of the animal) of the brain. Sixteen b values, ranging from 63 to 1898 s/mm², were used. DWI was acquired with a field of view of 25.6 × 25.6 mm, matrix size of 64 × 64 pixels, Δ of 10 milliseconds, echo time of 92 milliseconds, repetition time of 4000 milliseconds, and 2 excitations. The total acquisition time for producing the 8-slice ADC maps was ~2.1 minutes. The development of a “standard” (ie, acceptable) lesion was assessed by DWI after 15 minutes of MCA occlusion. DWI was repeated at 90 minutes after MCA occlusion to produce the ADC maps and ROIs. Data were transferred from the MR instrument to a computer workstation (100-MHz Iris Indigo R4000; Silicon Graphics Inc) for processing and subjected to a low-pass filter. Linear regression analysis was performed on a pixel-by-pixel basis to generate the ADC maps.

**Tissue Preparation**

After collection of the MRI data set at 90 minutes, the rats were rapidly removed from the magnet. While the animals were still under anesthesia, the chest and heart were quickly opened, a catheter was placed into the left ventricle, and 250 mL of 0.9% saline (USP Baxter) containing 1000 U of heparin was infused at 100 mm Hg inflow pressure into the ventricle and subsequently the vascular system. The time that elapsed between conclusion of the DWI measurement and start of infusion of the saline infusate was 2 minutes. Immediately thereafter, 250 mL of 4% paraformaldehyde in 0.2 mol/L PBS was infused; the morphological state therefore represented the system after ~92 minutes of focal ischemia. After this, the severed head was placed overnight in 4% paraformaldehyde. The brain was removed from the head and cut into 7 coronal, 2-mm-thick slices (referred to hereafter as slabs to distinguish them from the MRI slices) using a rat brain matrix (Activational Systems). These slabs were labeled from A (most rostral) through G (most caudal). Histological sections, 6 μm thick, were obtained from slabs A to C and E to G. Slab D was cut into 8 pieces (4 per hemisphere) that were marked on the H&E- and toluidine blue–stained histological sections. The criteria of Auer were used to judge irreversibility versus reversibility of damage.

**Quantification of Morphological Abnormalities**

Histopathology was evaluated in each ROI by examining 15 nonoverlapping microscopic fields on the H&E-stained sections with light microscopy at a magnification of ×400. For each microscopic field (area 52 455 μm²), the numbers of swollen astrocytes and of normal and shrunken neurons were measured by 1 or 2 computerized image analysis systems (either Global Laboratory Image; Data Translation Incorporated, or M5 System; Imaging Research Inc) and expressed as number per millimeter squared (frequency). Normal and shrunken neurons and swollen astrocytes were identified by their intrinsic morphological features as seen on the H&E-stained sections and presented in Results. With the M5 System, the perimeters of the neuronal cell bodies were traced on the images derived from the histological specimens, and somal area per neuron was calculated and recorded by neuronal type (normal or shrunken).

To seek ultrastructural evidence of irreversible injury, 40 electron micrographs were made of shrunken neurons from ROI-b (n = 20) and ROI-c (n = 20). The criteria of Auer were used to judge irreversibility versus reversibility of damage.
Variation of ADC Plus Frequency and Relative Size of Normal and Abnormal Neurons Among ROI

<table>
<thead>
<tr>
<th>ROI</th>
<th>ADC, ×10³ cm²/s</th>
<th>Neuronal Frequency, n/mm²</th>
<th>Area of Neuronal Soma, μm²/neuron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Normal</td>
</tr>
<tr>
<td>a</td>
<td>6.6±0.2</td>
<td>1053±78</td>
<td>991±64*</td>
</tr>
<tr>
<td>b</td>
<td>4.9±0.2</td>
<td>969±101</td>
<td>581±69*</td>
</tr>
<tr>
<td>c</td>
<td>3.6±0.3</td>
<td>957±64</td>
<td>382±37*</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=8.
*P<0.05 among a, b, and c (ANOVA with post hoc Bonferroni corrected t test).
†P<0.05 for normal vs shrunken neurons (t test).

Results

MRI Findings

In both hemispheres of the sham-operated rats and in the contralateral (nonischemic) hemisphere of the MCA-occluded rats, DWI intensities and ADC maps (Figure 1, ROI-a) were identical and normal. The contralateral ADC is used as the “control” in the subsequent presentation of data (Table, ROI-a).

On the ischemic side, regions of DWI hyperintensity were most prominent in the lateral caudoputamen, which had the lowest ADC values (ROI-c in Figure 1 and Table). In the upper frontoparietal cortex, ADC was 75% of control (ROI-b in Figure 1 and Table).

Morphological Abnormalities of Neurons

In the cortex and lateral caudoputamen of the nonischemic hemisphere and of sham-operated rats, most neurons appeared to be normal, having well-defined nuclei, prominent basophilic nucleoli, and clearly visible cytoplasm (Figures 2A and 3A). In these control areas, ∼6% of the neurons had well-stained nuclei and perikarya and diamond-shaped cell bodies. The mean somal area of these neurons was identical to that of normal neurons in ROI-a, ROI-b, and ROI-c (Table). Such dark, full-sized neurons are always seen in normal brain and are considered to be artifacts23; they are referred to hereafter as basophilic neurons (Table).

In the upper frontoparietal cortex, 40% of the neurons were shrunken and further characterized by darkly stained, speckled, triangular nuclei and a lack of perikaryal cytoplasm (shrunken neurons in Figure 2B and Table). Vacuoles, undoubtedly swollen, watery astrocytic processes, surrounded most, if not all, shrunken neuronal cell bodies in ROI-b. Furthermore, the cytosol of the shrunken neurons was more electron lucent, and the cisternae of the rough endoplasmic reticulum and Golgi apparatus were dilated (ultrastructural data not shown). The size (area per cell) of the shrunken...
neurons in ROI-b was significantly less than that of normal neurons (Table).

In the ischemic lateral caudoputamen, 60% of the neurons were elongated and shrunken (ROI-c in Table) with dark, highly condensed nuclei and scarcely any cytoplasm. Their cell bodies often were scalloped (Figure 2C) and surrounded by swollen astrocytic processes (Figure 3B). The shrunken neurons were smaller (Table) and more distorted in ROI-c than in ROI-b (Figures 2B and 2C). The frequency of shrunken neurons increased, ADC decreased (Figure 4). Of importance, swollen neurons were never seen in ROI-b or ROI-c.

At this early stage, the histological signs of irreversible injury (cytoplasmic eosinophilia, nuclear pyknosis, and karyolysis) were not evident among the neurons from the 2 ischemic regions. The ultrastructures of the normal-sized neurons in both ROI-a and the 2 ischemic regions were identical, and these neurons appeared unaffected by 90 minutes of ischemia. The shrunken neurons in ROI-b and ROI-c (Figure 3B) had continuous plasma, nuclear, and mitochondrial membranes and no flocculent material within their mitochondria; these ultrastructural features are indicative of potentially reversible injury.23

Structural Abnormalities of Astrocytes

Because of faint nuclear staining and little perikaryal cytoplasm, normal astrocytes do not stand out on H&E-stained sections (Figure 2A). This was the case, in general, for the cortex of sham-operated animals and in the contralateral hemisphere of MCA-occluded rats; a few astrocytes, however, had fairly well defined nuclei and were somewhat more prominent than the others (frequency in ROI-a 62/mm²). Ultrastructurally, all the astrocytes viewed in ROI-a presented slightly hydropic nuclei, scant cytoplasm, and dense, complex arrays of surrounding cellular processes (Figure 3C), that is, normal morphology, and did not resemble in the slightest the swollen ones in the 2 ischemic regions.

In the ischemic cortex and caudoputamen, many astrocytes with prominent nuclei and abundant perikaryal cytoplasm were seen on light microscopy (Figures 2B and 2C). The
electron microscopic data confirmed that these astrocytes were swollen (Figure 3D). Within such astrocytes, the heterochromatin was clumped around the edge of the nucleus, and the perikaryal space was wide, extremely lucent, and virtually devoid of organelles (Figure 3D). The frequency of swollen astrocytic cell bodies (light microscopic data) was significantly higher in the ischemic ROIs than in ROI-a \((P < 0.001)\) and was higher in ROI-c than in ROI-b \((P < 0.001)\). Last, ADC appeared to decrease as the frequency of swollen astrocytes increased (Figure 4), but this dependency seemed weaker than that between ADC and the frequency of shrunken neurons (Figure 4).

### Ischemic Changes in the Neuropil

The normal neuropil of the frontoparietal cortex of sham-operated rats and the contralateral cortex of MCA-occluded animals was dense and tightly packed with a myriad of delicate branching axon terminals, small dendrites, and astrocytic processes (Figure 5A). Normal mitochondria with tight cristae were scattered throughout the neuropil. Incidentally, microglial processes are not evident in normal neuropil and were not evident in the acute ischemic neuropil.

In marked contrast, swollen postsynaptic terminals (dendrites) and astrocytic processes were seen within the neuropil of ROI-b (Figure 5B) and ROI-c (Figure 5C). Swelling of individual terminals and processes appeared to be greater in the lateral caudoputamen (Figure 5C) than in the frontoparietal cortex (Figure 5B). The spaces of swollen dendrites and astrocytic processes were combined for each ROI to yield a single index of cytotoxic edema in the neuropil; this space was significantly greater in ROI-c than in ROI-b (Figure 6). Mean ADC fell as the space of neuropilar cytotoxic edema rose (Figure 6).

### Morphological Abnormalities of the Capillary Compartments

In sham-operated rats and contralateral cortex of MCA-occluded rats, the capillaries were round with open lumina and a modest cuff of astrocytic processes (Figure 7A). Thin, dark pericytes were often seen within the external basement membrane (example not shown).
In the 2 areas where ADC declined, the capillary-associated abnormalities included extensive swelling of pericytes and endothelial cells and chromatin clumping within their nuclei (Figures 7B to 7D). At this time and in these places, the endothelial cytoplasm was filled with swollen organelles and pinocytotic vesicles, and astrocytic foot processes were markedly swollen, creating virtual “lakes” between capillaries and parenchyma. At some places in ROI-c, the swelling of endothelial cells, pericytes, and pericapillary foot processes plus the accumulation of intraluminal debris reduced the lumen to no more than a narrow slit (Figure 7D).

The capillary luminal space, an indicator of patency, was significantly less in ROI-b and ROI-c than in ROI-a (Figure 8; \(P<0.05\)). In the 2 ischemic regions, the area of the endothelial nucleus (the largest part of this cell) was 60% to 90% greater than normal (Figure 8, \(P<0.05\)). The space of the pericapillary astrocytic foot processes was significantly larger in ROI-b and ROI-c than in ROI-a (Figure 8; \(P<0.01\)).

**Discussion**

**Acute Cellular Changes and ADC Reduction**

In the present work, the structural and ultrastructural data were obtained from tissue perfused and fixed in a way that should eliminate artifactual “dark, shrunken” neurons. The latter are thought to be produced by pressure generated through handling unfixed brain tissue and impaired aldehyde fixation.22,23 Accordingly, the dark, shrunken neurons reported herein (Table) are almost certainly the products of ischemic injury.25

During the first 10 to 30 minutes of occlusion with this model, the rates of blood flow assessed by perfusion-weighted imaging fall to \(\sim 50\%\) of control in areas such as ROI-c and remain at this level thereafter.26 Autoradiographic data indicate that local blood flow can be as low as 10% of normal within the block of tissue that constitutes the various ROIs (J.D.F., L. Wei, and T. Nagaraja, unpublished observations). During the 90 minutes of occlusion, at least some blood flows through most capillaries, but it may be that none
Showing some rat strain or regional differences, or both, in neurons at this acute stage have the potential to recover. 90 minutes of MCA occlusion. Accordingly, the shrunken gy22,25,27–30 were not observed in either ischemic region after ischemic ROIs were shrunken, and the frequency of shrunken astrocytic foot processes (PvAFP) per electron micrograph in the 3 ROIs after 90 minutes of MCA occlusion. The symbol and mean ADC for each ROI are indicated in the key. The differences between ROI-a and ROI-b and between ROI-a and ROI-c are significant (P<0.05) for each of the areas measured.

flows in collapsed capillaries such as that depicted in Figure 7D. In conclusion, our model yields incomplete, not complete, ischemia.

According to several studies,10,11,13–16 the reduction in ADC that occurs within minutes of the initiation of ischemia is caused by the movement of water from the extracellular to the intracellular space as ATP is depleted and homeostasis of ions and other osmotically active species (eg, glutamate) is compromised. The present findings demonstrate that the severity of ADC reduction within the territory supplied by the occluded artery correlated with not only swelling of astrocytes, dendrites, and endothelial cells but also shrinkage of the neuronal soma, a process that may consume energy.

All parts of the affected astrocytes (nucleus, perikaryon, and processes in the neuropil and around capillaries) were markedly swollen and watery after 90 minutes of occlusion. It is likely that most of the water taken up by astrocytes and dendrites came from the interstitium, but some may have come from shrunken neurons (Figure 3B). Capillary endothelial cells, pericytes, and foot processes were also edematous and may have absorbed some fluid from blood in the lumen.

In contrast to the widespread swelling of dendrites, the nuclei and perikarya of 40% to 60% of the neurons in the ischemic ROIs were shrunken, and the frequency of shrunken neurons as well as the degree of their shrinkage varied between ROI-b and ROI-c. In these 2 ischemic ROIs, the remaining neuronal cell bodies were normal in size (Table), which raises the question as to why some shrink and others do not.

Irreversibly injured neurons as defined by morphology22,25,27–30 were not observed in either ischemic region after 90 minutes of MCA occlusion. Accordingly, the shrunken neurons at this acute stage have the potential to recover. Showing some rat strain or regional differences, or both, in the progression to death, Garcia et al28 observed a few necrotic neurons in the preoptic area of Wistar rats after only 1 hour of focal ischemia.

To the best of our knowledge, an array of quantitative morphological results for various brain cells and their processes, such as those given Table and Figures 4, 6, and 8, has not been reported before for any model of cerebral ischemia. In the past (representative studies are cited for each case), neuronal shrinkage has been either scored in some simple way21,27,28 or described but not quantified,10,16 whereas swelling of astrocytic processes and dendrites in the neuropil has been included in measured areas of pallor or ischemic injury.21,28,31 In seemingly all instances, swelling of astrocytic cell bodies has simply been noted in passing.16,21

Restricted Diffusion of Water and ADC

The free diffusion coefficient of water at 37°C is 32×10^-6 cm²/s.32,33 The ADC of water measured in cerebrospinal fluid approaches that value.34 In normal brain tissue, ADC is ~20% of that in water.35 Regarding mechanism, the membranes of cells and their organelles are considered the major restrictors of water diffusion in brain.33–35 If these membranes were highly permeable to water, then water would be able to move as if there were no barriers to diffusion, and ADC would be ~25 to ~30×10^-6 cm²/s (this slight lowering is the result of the greater viscosity of the cytosol and interstitial fluid relative to water). The interstitial component of restricted diffusion is well known for brain and spinal cord. Numerous studies (see the review of Nicholson and Sykova35 for details) indicate that (1) the extracellular space (ECS) is ~0.2 mL/g tissue (20% in physiological notation) and continuous, (2) molecules up to the size of small proteins move through this space via diffusion (normally, there is little or no convection), and (3) for extracellular markers with molecular weights of <5000 (eg, inulin and PEG 4000), the rate of diffusion in brain tissue is ~40% of that in water. Regarding the latter, the square root of the water-to-brain diffusion coefficient ratio (1.6 for normal brain) equals the tortuosity (λ). Physically, tortuosity indicates the longer distance, ~60% for normal brain, a molecule must travel around cells relative to the direct path through them.35,36

With DWI measurements, protons both outside and inside cells are magnetically labeled. Within the 3 ROIs of this study, the average “diameter” of the normal neuronal soma is ~11 μm, whereas that of the normal astrocyte is much less, ~5 μm (Figures 3A and 3C, respectively). Most of the interior of brain cells is occupied by the nucleus; the rim of cytoplasm around the nucleus is only 1 to 2 μm thick for neurons and less than that for astrocytes (Figure 3). In general, the smaller organelles such as mitochondria and the astrocytic processes and dendrites are <1 μm wide. The number of intracellular compartments and membranes, their arrangement in series (nuclear and other organelle membranes within cell membranes), and the size of cells, organelles, and processes suggest that most of the magnetically tagged intracellular water remains within brain cells during DWI measurements.

When cells or some of their compartments swell, it would be expected that the apparent rate of water diffusion within...
the swollen structure would increase because of the greater distance between restrictive membranes. The opposite would be true when cells and cellular compartments shrink. Overall, intracellular diffusion is the product of the rates of diffusion within the water-containing compartments multiplied by their respective size, and variations in ADC are the weighted average of all the extracellular and intracellular changes.

Model of Normal and Reduced ADCs in Brain

The following modeling will be used to (1) obtain an estimate of the normal effective intracellular diffusion coefficient, (2) illustrate the relationship among lowered ADC, loss of extracellular fluid, increase in tortuosity, cellular swelling, and neuronal shrinkage, and (3) suggest that neuronal shrinkage plays an appreciable role in acute, severe lowering of ADC. This analysis is based on a simple model originally proposed by Benveniste et al. The model consists of 2 parallel pathways for water diffusion (1 extracellular and 1 intracellular) and weights the contributions of extracellular and intracellular diffusion by the size of each compartment relative to total tissue water. In past modeling, the extracellular component of ADC was set equal to the free diffusion coefficient of water at 37°C. Because the extant data argue against this (briefly reviewed earlier), it will not be used in our modeling.

The following assumptions will be made for estimation of the effective diffusion coefficient of intracellular water and for the subsequent modeling. First, the extracellular space will be considered to be tortuous; the effective diffusion coefficient for water in it will, therefore, be assumed to be 40% of the free diffusion coefficient (eDex = 12.8 × 10⁻⁶ cm²/s = 0.4 × 32 × 10⁻⁶ cm²/s). Second, 25% of brain water will be considered extracellular and 75% will be considered intracellular (a review of the literature suggests that these are better estimates than the 20:80 split used previously). Third, for the acute case, brain volume will be assumed to be constant, and the volume decreases of some compartments will be matched by the volume increase of others. Finally, the assumption of no water exchange between extracellular and intracellular spaces over the very short interval of measurement (40 milliseconds) is implicit to the parallel pathway model. The working equation is ADC = (ECW × eDex) + (ICW × eDic), where ECW and ICW are the extracellular and intracellular water spaces, respectively, and eDex is the effective diffusion coefficient of water within brain cells. Insertion of the normal gray matter ADC (6.6 cm²/s; Table) and the assumed values of eDex and the 2 water spaces into the equation yields eDic = 4.5 × 10⁻⁶ cm²/s. With this estimate of eDic and a DWI measurement time of 40 milliseconds (Δ), the root-mean-square distance of diffusion (the square root of eDic × Δ) is 4.2 μm, which is somewhat less than the somal diameters of neurons (11 μm) and astrocytes (5 μm).

To model the dependency of the decline in ADC on decrease in extracellular space, increase in interstitial tortuosity, and change in intracellular volume, the following case will be considered: ECS of 5%, ECW of 6%, ICW of 94%, λ of 2.2, and eDex = 6.6 × 10⁻⁶ cm²/s. These values have been reported for severe ischemia and complete anoxia and are the most extreme reported to date. Insertion of these values and the mean ADC for ROI-b (Table) into the working equation yields a value of eDic equal to 4.8 × 10⁻⁶ cm²/s, which is slightly larger than that calculated here for normal brain (ROI-a). To reiterate, the change in ADC is the sum of the processes that lower diffusion within the tissue (shrinking extracellular and neuronal spaces and increasing λ) and raise it (swelling astrocytic, dendritic, and endothelial compartments). In ROI-b, the effects of neuronal shrinkage and cytotoxic edema may therefore be virtually equal in size but opposite in direction and produce little or no net increase in eDic.

Repeating this calculation with the ADC measured for ROI-c (Table) produces a value for eDic of 3.6 × 10⁻⁶ cm²/s, which is 20% to 25% lower than that estimated for ROI-a and the case just modeled for ROI-b. In ROI-c, the effects of neuronal shrinkage may outweigh those of cellular swelling and thereby lower eDic and drop the root-mean-square distance of diffusion from 4.2 to 3.8 μm.

In conclusion, the cellular term in the working equation (ICW × eDic) mostly certainly overestimates the system by not only combining neurons and glia but also lumping together many intracellular compartments, surface areas, intracellular diffusion coefficients, and membrane permeabilities to water. The modeling of acute changes was based on the extreme case of interstitial shrinkage, but the results would be very similar if less extreme conditions had been used. Despite these qualifications, the present findings and modeling indicate that DWI is fairly sensitive to perturbations in water compartmentation and that neuronal shrinkage probably is as important as cytotoxic edema in setting ADC during acute ischemia.

Acknowledgments

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References

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