A Model for Multiparametric MRI Tissue Characterization in Experimental Cerebral Ischemia With Histological Validation in Rat

Part 1

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Background and Purpose—After stroke, brain tissue undergoes time-dependent heterogeneous histopathological change. These tissue alterations have MRI characteristics that allow segmentation of ischemic from nonischemic tissue. Moreover, MRI segmentation generates different zones within the lesion that may reflect heterogeneity of tissue damage.

Methods—A vector tissue signature model is presented that uses multiparametric MRI for segmentation and characterization of tissue. An objective (unsupervised) computer segmentation algorithm was incorporated into this model with the use of a modified version of the Iterative Self-Organizing Data Analysis Technique (ISODATA). The ability of the model to characterize ischemic tissue after permanent middle cerebral ischemia occlusion in the rat was tested. Multiparametric ISODATA measurements of the ischemic tissue were compared with quantitative histological characterization of the tissue from 4 hours to 1 week after stroke.

Results—The ISODATA segmentation of tissue identified a gradation of cerebral tissue damage at all time points after stroke. The histological scoring of ischemic tissue from 4 hours to 1 week after stroke on all the animals was significantly correlated with ISODATA segmentation ($r=0.78$, $P<0.001$; $n=20$) when a multiparametric (T2-, T1-, diffusion-weighted imaging) data set was used, less correlated ($r=0.70$, $P<0.01$; $n=20$) when a T2- and T1-weighted data set was used, and not correlated ($r=-0.12$, $P>0.47$; $n=20$) when only a diffusion-weighted imaging data set was used.

Conclusions—Our data indicate that an integrated set of MRI parameters can distinguish and stage ischemic tissue damage in an objective manner. (Stroke. 2001;32:943-949.)

Key Words: cerebral ischemia, focal | computer-assisted image processing | diffusion imaging | magnetic resonance imaging | signal processing, computer assisted, ISODATA | stroke, acute | stroke classification | tissue signature

MRI parameters within an ischemic lesion are time dependent and heterogeneous, and it is unlikely that a single MR parameter can characterize the complexity of cerebral tissue.1–5 In clinical practice, diffusion-, T2-, and T1-weighted images (DWI, T2WI, T1WI, respectively) are acquired during the progression of stroke at most medical centers. An integration of these MRI data may provide complementary information about the status of the tissue.

MRI generates multiparametric data because of its unique ability to form images influenced by different types of tissue parameters (ie, proton density–weighted imaging, T2WI, T1WI, and DWI).6 In addition, by using these MR images, an n-dimensional feature space can be constructed by plotting each MR image on a separate axis.7 In particular, unsupervised segmentation methods, such as K-means and fuzzy c-means, can be applied to multiparametric MRI data for exploratory clustering and analysis. However, the difficulty with K-means and fuzzy c-means segmentation methodologies is that the number of tissue clusters should be known a priori.7 In practice, the number of tissue clusters is not usually known during the evolution of cerebral ischemia because the ischemic tissue damage is...
heterogeneous and time dependent. These challenges require an algorithm that can adjust the number of tissue clusters in an iterative fashion and incorporate multiple MRI parameters.

To address the difficulty of characterizing ischemic brain tissue damage independent of time, a modification of a previously reported tissue signature model for classifying ischemic tissue damage was implemented. This supervised tissue signature model required an operator to define a rectangular box on a scatterplot using the mean and SD of tissue signature model required an operator to define a rectangular box on a scatterplot using the mean and SD of water (ADC) maps. The need for supervision to determine normal tissue from T2 and apparent diffusion coefficient of tissue was a drawback of the model, and validation of this model was incomplete.

We have developed an unsupervised segmentation algorithm that incorporates multiparametric MRI. The algorithm is based on an Iterative Self-Organizing Data Analysis Technique, commonly referred to as ISODATA. ISODATA is an iterative, multistep process that assigns the input data into a set of clusters. This study presents histological validation of a novel vector signature model based on ISODATA segmentation of abnormal from normal tissue.

Materials and Methods

Theory

Angle Model Classification of Tissue Clusters

We hypothesized that different tissue classes in brain, such as white matter, gray matter, cerebrospinal fluid, and abnormal tissue, will exhibit distinct characteristic MR signal intensities and that these different MR signals can provide a way to classify the tissue. Therefore, to classify ischemia in tissue, a novel model of tissue signatures was developed on the basis of the angular separation of abnormal tissue from normal tissue by the use of vectors. The tissue signature vector is a mathematical descriptor to identify and/or classify tissue types in n-dimensional feature space created from the multiparametric MRI data, as described below. The tissue signature vector is defined as follows:

\[
S_{ij} = [S_{ij}, S_{2ij}, \ldots, S_{nij}]^T
\]

where \( S_{ij} \) is the k-th element of the i-j-th pixel vector, ie, the mean gray level of the i-j-th pixel in the k-th image in the image sequence for each cluster, and \( T \) is the transpose of the matrix. Typical MRI data used in this study are shown in Figure 1. Each of the MR images represents a dimension in feature space, and in our study a 5-dimensional feature space is used for the ISODATA model. A representation of tissue signature vectors defining different tissues is demonstrated in Figure 2A. The corresponding ISODATA theme map (Figure 2B) is constructed from tissue clusters and tissue signature vectors that represent the centroid for each tissue cluster (see below).

A feature space or variable space can be constructed by plotting the signal intensity of each MR image on a separate axis, which, in other terms, is a multidimensional histogram of the MR image signal intensity. To demonstrate the concept of feature space and different tissue clusters created from 3 MR images with the angular separation between the tissue clusters, a representation of a 3-dimensional feature space is shown in Figure 2C using only T2WI, T1WI, and DWI as input parameters. Using the distribution of the tissue clusters in feature space defined by ISODATA, a tissue signature vector \( S \) is created for each cluster, allowing the different tissue signature vectors to be tested for similarity. We hypothesize that if tissue clusters are close together in the

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Representative acute (4 to 8 hours) MR data used for the ISODATA algorithm in rats subjected to experimental cerebral ischemia. A, Proton density–weighted image (TE=30 ms); B, T2WI (TE=90 ms); C, T1WI; D and E, DWI (D, b=600 s/mm^2; E, b=800 s/mm^2).

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Demonstration of tissue signature vectors for different tissue types on MR data from the animal in Figure 1. A, Signature vectors are defined for each separate tissue class and assigned to the cluster that closely resembles its vector elements in the ISODATA algorithm. B, A representative ISODATA theme map from the tissue signature vectors defined from the centroid of each tissue cluster is shown. The dark blue color encompassing the contralateral hemisphere and frontal and parietal cortex of the ipsilateral hemisphere represents normal tissue. The various colored regions (light blue to red) within the ipsilateral hemisphere represent abnormal tissue clusters. Cerebrospinal fluid (red) was segmented as a separate tissue class. C, A representation of the 3-dimensional feature space formed by T1, T2, and DWI (the 3-dimensional feature space is shown for easy visualization). The angle separation model is demonstrated with the distribution of tissue clusters in the 3-dimensional feature space. Angles are calculated by the inner product between the normal tissue and abnormal tissue clusters using each cluster’s tissue signature vector, as shown by \( \theta_1 \) and \( \theta_2 \). Each axis represents the signal intensity distribution for each MR image.
feature space, then their coordinates and measurements will be similar, and the angles between tissue signature vectors will be small. On the other hand, if the clusters are far apart in the feature space, this would imply that the measurements are different, and the angular separation will be large.

Within the context of cerebral ischemia, in regard to tissue that has undergone less severe ischemic damage, (1) the tissue will exhibit less histological damage and a low histological score (see below), and we hypothesize that (2) the tissue will have a small or no difference in angle from the normal tissue. As the severity of ischemia increases, there is a corresponding increase in histological (ie, morphological) damage to the tissue, as indicated by both the angular separation between tissue clusters and histological scoring.

**The ISODATA Technique**

The ISODATA technique is an unsupervised segmentation method related to the K-means algorithm and has the ability to adjust the number of clusters. The main advantage of the ISODATA method is that it requires no initial training or a priori knowledge of the exact number of tissue classes before segmentation.

**ISODATA Parameter Selection for Experimental Focal Ischemia in Rat**

For experimental stroke studies using small animals, parameters are initialized as follows. We set the number of initial clusters (K) to K=15 and desired clusters to K=5. Note that this number (K=5 for desired clusters) will be automatically adjusted by ISODATA if the algorithm determines that the number of desired clusters is inadequate to represent the structure of the data. Other parameters are as follows: the minimum number of pixels in a cluster: \( \theta_h = 5 \); the SD of gray matter: \( \theta_g \); the Euclidean distance between normal tissue in brain: \( \theta_i \); ie, white and gray matter; the maximum number of cluster pairs to lump together in 1 iteration: \( I = 1 \); the maximum number of iterations: \( I = 100 \); and the cluster center constant: \( \gamma = 0.25 \sigma_{max} \), where \( \sigma_{max} \) is the maximum element for each SD vector of the cluster.

**The ISODATA Algorithm**

Our modified ISODATA algorithm consisted of the following steps (there are a total of 14 steps in the ISODATA algorithm; for a complete description, see Jacobs et al). 1) The clustering parameters are included in the program. 2) The MRI data is partitioned into random clusters. 3) Cluster centers are determined for each cluster. 4) Intra-Euclidean distances (InAD) are calculated on a pixel-by-pixel basis between each pixel and its cluster center. 5) Inter-Euclidean distances (IED) are calculated between each of the cluster centers. 6) Splitting and merging of clusters are performed on the basis of InAD and IED. Clusters that have a large InAD are split, ie, the cluster has a large SD. Clusters the have a small IED are merged. 7) Steps 5 and 6 are repeated until the algorithm converges or reaches the maximum number of iterations allowed. Convergence is defined as the minimization of the variance of the clusters between iterations. After convergence is reached, the tissue clusters are formed into a theme map (Figure 2B). A theme map is a color-coded composite image that reflects the different types of tissue classes that were segmented from the data set.

With the use of the ISODATA tissue clusters within the ischemic hemisphere, abnormal tissue signature vectors are constructed. A normal tissue signature vector, ie, the reference vector, representing the corresponding normal tissue in the contralateral hemisphere, is also constructed. Then the angular separation between each tissue signature vector is calculated with the use of the inner product between vectors; this is demonstrated in Figure 2C. The angle measurement may provide an index for comparison with the histological score.

**Experimental Animal Model**

All studies were performed in accordance with the institutional guidelines for animal research under a protocol approved by the Institutional Care of Experimental Animals Committee. Male Wistar rats (n=20) weighing 270 to 310 g were anesthetized with 3.5% halothane and maintained with 0.75% to 1.5% halothane in 70% \( \text{N}_2 \text{O} \) and 30% \( \text{O}_2 \) with the use of a face mask. Rectal temperature was controlled at 37°C with a feedback-regulated water-heating pad. Rats were subjected to permanent occlusion of the middle cerebral artery (MCA) (n=20) by a method of intraluminal vascular occlusion. This method produces a focal infarct in the striatum, ie, caudate putamen and globus pallidus, that may extend into the cortex. Ischemic animals were classified on the basis of time of euthanasia after stroke: acute (4 to 8 hours; n=5), subacute (16 to 24 hours; n=9), and chronic (48 to 168 hours; n=6).

**MRI Acquisition**

After MCA occlusion, the animal was placed in the magnet, and MRI data sets (DWI, T2WI, and T1WI) were acquired on a 7-T, 20-cm-bore, superconducting magnet (Magnex Scientific Inc) interfaced to a SMIS (Surrey Medical Imaging Systems Ltd) console. A 5-cm–internal diameter birdcage radio frequency coil and 12-cm-bore actively shielded gradient coil set capable of producing magnetic field gradients up to 20 G/cm were used. The head of the animal was secured with stereotaxic ear bars to reduce motion during the experiment. Once the animal was placed inside the magnet, 2 orthogonal interleaved fast low-angle shot (FLASH) images (coronal and sagittal planes) were acquired for accurate positioning of the animal, as previously described.

During MRI measurements, anesthesia was maintained with 0.75% to 1.5% halothane in a 70% \( \text{N}_2 \text{O} \) and 30% \( \text{O}_2 \) gas mixture. Rectal temperature was monitored and controlled with a feedback-controlled water bath. MRI studies were performed on animals after MCA occlusion at acute, subacute, and chronic postischemia time points. Multislice (2-mm-thick contiguous slices) DWI, T2WI, and T1WI were obtained with a 128×128 image matrix with a 32-mm field of view. DWI were acquired by the pulsed gradient, spin-echo method described by Le Bihan et al, with repetition time/echo time \((\text{TR}/\text{TE}) = 1500/40\) ms and diffusion-weighted gradients \((7\) slices, with incremented b values of \(0, 200, 400, 600,\) and \(800\) s/mm\(^2\); number of excitations \([\text{NEX}] = 2\) applied along the z axis. T2WI were acquired by a multiecho sequence \((7\) slices; \(\text{TR}/\text{TE} = 3000/30, 60, 90,\) and \(120\) ms; \(\text{NEX} = 1\)), and a T1WI inversion recovery image \((5\) slices; \(\text{TR}/\text{TE} = 6000/30\) ms; inversion time = 750 ms; \(\text{NEX} = 1\)) was also obtained.

**MRI Image Preprocessing and Analysis**

MR image analysis was performed with a SUN UltraSPARC2 workstation (Sun Microsystems Inc). All MR images were reconstructed with a 128×128 matrix with the use of in-house software and subsequently processed with Eigentool image analysis software.

Preprocessing on the MRI data consisted of several steps that included subimaging, inhomogeneity correction, and noise reduction. Subimaging of the intracranial volume was done to segment the image background, skull, and scalp from brain tissue. After subimaging, an inhomogeneity correction method was applied to the MRI data set. Finally, image noise was reduced with the use of a nonlinear restoration filter that reduces white noise while preserving edges and partial volume information. Maps of ADC and T2 were created for each time point using a least-squares fit from the slope of the signal intensity on a pixel-by-pixel basis.

**MRI Data Analysis**

Coregistration and warping of the MRI to histology were accomplished by a previously reported 2-step methodology. After coregistration and warping, 3 different sets of MRI image data were used in the ISODATA algorithm: \((1) 5\) DWI; \((2) 2\) T2WI \((\text{TE} = 30, 90\) ms), T1WI, and \(2\) DWI \((b=600, 800\) s/mm\(^2\)); and \((3) 4\) T2WI and 1 T1WI. These choices of MR parameters were selected on the basis of preliminary studies of both laboratory animals and humans demonstrating that these sets of MR parameters define and characterize stroke over time. Each of these data sets was used as an input into the ISODATA algorithm to create 3 different theme maps (Figure 2B). From each of the theme maps, ISODATA lesion tissue clusters were
defined, and a region of interest (ROI) was automatically created. The ISODATA ROIs were overlaid onto ADC and T2 maps to obtain quantitative values for each tissue cluster. Similar ROI analysis was performed on the normal tissue clusters. The values of the ADC and T2 from the total lesion area within the ipsilateral hemisphere were normalized to the contralateral hemisphere and expressed as ratios. Comparison of the ISODATA ROI analysis with ADC and T2 maps enabled us to address the following question: Does the angle model segment ischemic and/or infarcted tissue?

### Histopathological Analysis

#### Tissue Processing

All animals were killed immediately after imaging for histopathological evaluation. Animals were deeply anesthetized with ketamine (44 mg/kg) and xylazine (13 mg/kg) by intraperitoneal injection and were transcardially perfused with heparinized saline and 10% neutral buffered formalin. The brain was removed and immersed in the same fixative overnight. Fourteen coronal blocks of brain tissue were cut at 1-mm intervals with the use of a rat brain matrix. The tissue was processed and embedded in paraffin. Paraffin sections from each block (6 μm thick) were cut and stained with hematoxylin and eosin (H&E) for evaluation of ischemic cell damage.

#### Regional Light Microscopy Analysis

The coregistered/warped ISODATA-defined lesion areas were overlaid onto the corresponding H&E histological sections, and 2 to 4 fields of view (392×280 μm²) in the ipsilateral hemisphere and the homologous areas in the contralateral hemisphere were digitized under a light microscope with a 40 objective lens (Olympus BX40) with a charge-coupled device camera (Hitachi RP-111) interfaced with Global Laboratory image analysis system (Data Translation).

Each image was analyzed with an MCID image analysis system (Imagez Research), and the coordinates for each image were recorded. A value of the mean gray scale of the entire image and 2 SDs of the mean was used to measure the number of cells and vacuolization. In addition, for measurement of vacuolization, we inverted images to obtain improved gray scale visualization. These values were selected on the basis of our preliminary study, in which we manually counted numbers of cells for each image and then compared the numbers of cells counted by the MCID with several different thresholds of gray scale intensity within the same image. We compared the number of cells obtained from both manual and computer counting of >200 images using the mean gray scale value from the entire image and 2 SDs. Differences in the numbers of cells between these 2 methods were <5%. In addition, 2 blinded observers (Z.G.Z., A.V.G.) measured the number of cells in same image (n=102) with these values, with an interobserver difference of 1.25%. Changes in vacuolization are presented as a percentage of the field, in which areas of vacuoles were divided by total field area.

#### Histological Grading Measurements

On the basis of prior studies in our laboratory,12,21 the criteria for ischemic neuronal damage were the presence of scalloping at the cyttoplasmic border, triangular shrunken neurons (acute ischemic neuronal damage), and eosinophilic and ghost neurons (chronic ischemic damage). Because the MCID image analysis system could not differentiate these morphological changes, we combined numbers of cells quantified by the MCID with visual observation of ischemic neuronal damage under the light microscope on the basis of the criteria outlined above. To reflect the heterogeneous nature of the ischemic lesion evolution, we developed a grading scale ranging from 0 to 10 for the present study, with no neuronal damage scored 0 and the most severe neuronal damage scored 10. Neuronal shrinkage and morphological alterations were all considered potentially reversibly damaged tissue and were scaled as follows: 0, no neuronal damage; 1, <20%; 2, 21% to 50%; 3, >50%; and 4 to 5, combinations of 2 and 3 such that 1 is added to the score if >50% reduction in number of total cells is seen compared with the contralateral hemisphere or 2 is added for >50% reduction in the number of total cells compared with the contralateral hemisphere.

Neuronal necrosis, eosinophilia, red neurons, and ghost neurons were considered potentially irreversible damaged tissue, assigned the following scale: 6, <20%; 7, 21% to 50%; 8, >50%; and 9 to 10, combinations of 7 and 8 such that 1 is added to the score if <50% reduction in number of total cells is seen compared with the contralateral hemisphere or 2 is added for >50% reduction in the number of total cells compared with the contralateral hemisphere.

Histological grade accounts for both morphological changes in neurons, characterizing reversible and irreversible neuronal damage, as well as parenchymal cell loss, which encompasses total cell loss of neurons and glia.

#### Statistical Analysis

Paired t tests were used to determine statistical significance between the histological measurements determined from each ISODATA cluster and corresponding homologous contralateral regions with the null hypothesis that there is no association between the histological score and the angular measurement. Linear regression analysis was performed to correlate the angle measurements with the histological grading of the ISODATA-defined tissue clusters. All parametric map values are presented as mean±SD. Statistical significance was assigned for P<0.05.

### Results

The ISODATA-defined lesion revealed ischemic cellular damage localized to the ipsilateral hemisphere. A representative ISODATA theme map at 4 to 8 hours after stroke is shown in Figure 3. At all time points, the ISODATA theme map had at least 2 distinct zones within the ischemic hemisphere. Ischemic cell damage changed over time. After acute (4 to 8 hours) stroke, ischemic cellular changes consisted of shrunken and triangular neuronal cell bodies with neuronal perikarya and perineuronal vacuolation (Figure 3A, 3C, 3E). At the subacute (16 to 24 hours) time points, ischemic neuronal damage was seen as cytoplasmic eosinophilia (red neurons) mixed with ischemic neuronal damage, as described above.12,21 At the chronic (48 to 168 hours) time points after cerebral ischemia, abundant ghost neurons and pan necrosis were noted within the ISODATA-defined clusters (Figure 4A and 4C). These histological findings are consistent with previously reported histological characteristics of evolving cerebral ischemic cell damage.12,21

Representative ADC and T2 values from the multiparametric data set (T2WI, T1WI, and DWI) are shown in Table 1. The values obtained from the overlaid ISODATA regions onto the ADC and T2 maps were consistent with previously reported studies of ischemic regions after stroke.4,22 These data suggest that the ISODATA changes reflect the evolution of ischemic brain tissue injury from normal to final infarction.

### ISODATA Model Using T2WI, T1WI, and DWI

Correlations of angle measurements with histological scoring from the multiparametric set consisting of 2 T2WI, 1 T1WI, and 2 DWI using the ISODATA model are summarized in Table 2. Compared with the DWI and T2WI/T1WI data sets (see below), the multiparametric data set that combined the T2WI, T1WI, and DWI exhibited the highest correlation between the angle measurements and histological scoring (r=0.78, P<0.007; n=20). The average angle between abnormal and normal tissue was 7.8±1.8° (range, 6.1° to 16.5°), with histological score of 4.2±1.4 (range, 2.0 to 7.0), 4 to 8 hours after stroke. This angular separation continued to

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increase to 15.4±8.8° (range, 6.3° to 37.4°), with histological score of 7.1±2.6 (range, 3.0 to 10.0), at the chronic time point (48 to 168 hours).

**ISODATA Model Using DWI**

Acutely, the ISODATA model using the DWI data set exhibited a significant correlation ($r=0.79$, $P<0.02$) between the angle measurement model and the histological score of the tissue. However, no correlation with the histological score was seen at subacute and chronic time points ($r=-0.05$, $P>0.68$; $r=-0.04$, $P=0.90$, respectively). Overall, the DWI ISODATA angle measurements failed to correlate with the histological scoring on all animals ($r=0.12$, $P>0.47$; $n=20$). These data are summarized in Table 2. The average angle between abnormal and normal tissue was 5.6±2.8° (range, 2.2° to 12.8°), with a histological score of 3.8±0.48 (range, 2.0 to 7.0) at 4 to 8 hours after ictus. The angular separation then decreased to 2.9±1.7° (range, 0.68° to 6.9°) at the chronic time points, with corresponding histological scores of 7.2±0.74 (range, 3.0 to 10.0).

**Figure 3.** The warped ISODATA with the lesion boundary overlaid onto the histological H&E-stained section from representative acute (4 to 8 hours) animal after stroke. A through F, Magnified regions (×40) were taken from the caudate putamen (I1-C1), pyriform cortex (I2-C2), and preoptic areas (I3-C3). D, E, and F, Normal tissue was noted within the contralateral regions: C1, C2, and C3. A, B, and C, The histological morphology within the ISODATA regions I1, I2, and I3 consisted of acute ischemic cellular damage. A and C, These histological changes were visualized as shrunken and triangular neuronal cell bodies with neuronal perikarya, surrounded by vacuoles, and had an angular separation of 14.7° from normal tissue and a histological score of 6. In contrast, B had a smaller angular separation of 7.3° and a lower histological score (3). Tissue vacuolation corresponding to both swollen astrocytes and neurons was most prominent in the striatum (I1 and I3). CSF indicates cerebrospinal fluid. Bar=50 μm.

**Figure 4.** A warped multiparametric ISODATA with the lesion boundary overlaid onto the histological H&E-stained section from representative chronic (48 to 168 hours) animal after stroke. A through D, Magnified regions (×40) taken from the frontoparietal cortex (I1-C1) and preoptic areas (I2-C2) areas. B and D, In contralateral regions, C1 and C2, normal tissue was noted. A, Ischemic cellular damage within I1 defined by ISODATA consisted of irreversible cellular changes such as shrunken, dark, and triangular neurons with eosinophilia. Increased perineuronal and tissue vacuolation was evident within this region. The angular separation was 12.2° from normal tissue. C, In region I2, similar but more extensive cellular damage with tissue loss was detected, and it had a larger angle (22.1° from normal tissue). CSF indicates cerebrospinal fluid. Bar=50 μm.
TABLE 1. Ratios of ADC and T2 Maps as Defined by Multiparametric ISODATA With Angle, Histological Score, and Correlation Between Angular Separation and Histological Score

<table>
<thead>
<tr>
<th>Time</th>
<th>Angular Separation</th>
<th>Histological Score</th>
<th>Correlation With Histological Score</th>
<th>ADCr</th>
<th>T2r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute (4–8 h)</td>
<td>7.8±1.8°</td>
<td>4.2±1.4</td>
<td>0.76*</td>
<td>0.63±0.19</td>
<td>1.08±0.06</td>
</tr>
<tr>
<td>Subacute (16–24 h)</td>
<td>11.5±5.5°</td>
<td>5.7±1.9</td>
<td>0.78*</td>
<td>0.73±0.26</td>
<td>1.48±0.31</td>
</tr>
<tr>
<td>Chronic (48–168 h)</td>
<td>15.4±8.8°</td>
<td>7.1±2.6</td>
<td>0.76*</td>
<td>1.07±0.24</td>
<td>1.54±0.04</td>
</tr>
</tbody>
</table>
| ADCr indicates ADC ratio; T2r, T2 ratio. *P<0.01.

ISODATA Model Using T2WI and T1WI

The ISODATA model using 4 T2WI and 1 T1WI showed a correlation between the angle measurements and histological scoring (r=0.70, P<0.01; n=20; Table 2). The average angle between abnormal and normal tissue was similar to that in the multiparametric data set (data not shown).

Discussion

Using an unsupervised segmentation algorithm, we have developed, tested, and validated a novel MR tissue signature model of ischemic cell damage based on the angular separation between abnormal and normal tissue clusters in a rat model of experimental focal cerebral ischemia. The tissue signature model was developed with the use of tissue signature vectors from different classes of tissues obtained from ISODATA cluster analysis in conjunction with multiparametric MRI data. Histological scoring of ROIs identified from the coregistered/warped segmented ISODATA tissue clusters overlaid onto H&E histological slides was performed to validate the model. The histological score of the ischemic tissue at each time point correlated significantly with the angular separation of abnormal tissue when the multiparametric MR data set was used, suggesting a truly independent measure of the state of the tissue. Note that the tissue clusters are separated in n-dimensional feature space by the Euclidean distance classifier used in ISODATA. This provides an additional criterion to ensure that there is no overlapping of clusters and may be used for further classification of ischemic tissue.

TABLE 2. Correlation Coefficient Between Histological Score and Each Different Input MR Data Set Used in the ISODATA Angle Model

<table>
<thead>
<tr>
<th>MR Image Sets</th>
<th>Acute (4–8 h)</th>
<th>Subacute (16–24 h)</th>
<th>Chronic (48–168 h)</th>
<th>All animals (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 DWI</td>
<td>0.79</td>
<td>(P&lt;0.02)</td>
<td>(P&lt;0.008)</td>
<td>(P&lt;0.0007)</td>
</tr>
<tr>
<td>4 T2WI, 1 T1WI</td>
<td>0.73</td>
<td>0.70</td>
<td>0.78</td>
<td>0.72</td>
</tr>
<tr>
<td>2 T2WI, 1 T1WI, 2 DWI</td>
<td>0.76</td>
<td>(P&lt;0.002)</td>
<td>(P&lt;0.0002)</td>
<td>(P&lt;0.0003)</td>
</tr>
<tr>
<td>(P&lt;0.90)</td>
<td>(P&lt;0.01)</td>
<td>(P&lt;0.007)</td>
<td>(P&lt;0.0001)</td>
<td>(P&lt;0.001)</td>
</tr>
</tbody>
</table>

Values are correlation coefficients (r). Of the 3 MRI data sets used in the ISODATA segmentation, the multiparametric set that included DWI, T2WI, and T1WI consistently produced better correlation with the histological scoring than the other data sets at each time point. Only the DWI input data set exhibited a higher correlation between the angular separation and the histological score at the acute stage of stroke, but it failed to correlate with the status of the tissue at later time points. DWI measures cytotoxic edema and is very sensitive to the acute changes in tissue water (ie, cytotoxic edema). During the transition from the subacute to chronic stages, the ADC pseudonormalizes and results in a lack of correlation between the DWI ISODATA model and histological scoring.

The findings of this study (1) support the hypothesis that a multiparametric approach is needed to accurately identify and stage ischemic tissue; (2) show the versatility of the modified ISODATA algorithm, which incorporates several different MRI parameters to segment multiple tissue classes; and (3) accurately define the state of the tissue in this model of experimental stroke.

The ISODATA model overcomes several limitations of our previously reported tissue signature model. First, we have eliminated the use of rectangles to define tissue classes by incorporating an unsupervised cluster analysis technique. Cluster analysis can recognize structures within a data set, assuming that there is some type of structure that can be grouped into different tissue classes. In MRI data, this assumption of different classes is well founded, since there is a clear distinction between cerebrospinal fluid and white and gray matter in brain. In addition to the normal tissue classes, pathological disease states can result in additional tissue classes. Second, the number of MRI images needed for identification of the ischemic tissue is arbitrary and can be defined by the user. By increasing the dimensionality of the model, increased separation of different tissues can be realized. Finally, the ISODATA model provides an objective classification of the tissue clusters independent of time. This is supported by the histological scoring of the tissue clusters coupled with the morphological changes noted within each subregion of the ischemic lesion. The results of this study show the advantages of using multiparametric MRI data to characterize ischemic tissue.

Recent reports have discussed a multiparametric imaging approach using T2WI, proton density, ADC, and bolus tracking cerebral blood flow estimates incorporated into an
unsupervised segmentation methodology in different models of experimental cerebral ischemia with and without reperfusion. Carano et al demonstrated that the unsupervised K-means algorithm outperformed supervised segmentation methodologies, including fuzzy methods for segmentation of ischemic tissue approximately 3 hours after stroke, giving the best classification rate and correlation with 24-hour 2,3,5-triphenyltetrazolium chloride–stained histological sections. Our results from the present study support these findings and emphasize the need for a multiparametric MR approach to characterize the complex evolution of cerebral ischemia. However, there are differences between our multiparametric approach and those reported.

First, ISODATA is related to K-means, but the advantage of ISODATA is that it has additional splitting and merging techniques to adjust the numbers of initial clusters selected in the data. This adjustment of the number of tissue clusters is important because the number of tissue clusters is not known during the evolution of cerebral ischemia. Second, in this study the animals were imaged and killed at specific time points with the use of H&E staining to identify the histological lesion area and morphological characteristics. Third, coregistration and warping was used to overlay the ISODATA regions onto the H&E histological slides. This allowed for a direct mapping between ISODATA and histology. It must be stressed that these reports, coupled with our studies, provide complementary data for the difficult task of characterizing stroke and developing methods to provide automated computer-assisted techniques that can be used in the clinical setting.

The addition of perfusion data into the ISODATA model would likely increase the performance of our ISODATA model in identification and characterization of acute stroke “tissue at risk” and is currently under active investigation. The application of this tissue characterization method to clinical stroke is currently under active investigation. The application can be used in the clinical setting.

Conclusion

We have demonstrated that integration of multiparametric MRI data in the ISODATA angle model provides useful information about the histological status of ischemic tissue.

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