Reperfusion in a Gerbil Model of Forebrain Ischemia Using Serial Magnetic Resonance FAIR Perfusion Imaging

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Background and Purpose—Existing methods for the quantitative measurement of the changing cerebral blood flow (CBF) during reperfusion suffer from poor spatial or temporal resolution. The aim of this study was to implement a recently developed MRI technique for quantitative perfusion imaging in a gerbil model of reperfusion. Flow-sensitive alternating inversion recovery (FAIR) is a noninvasive procedure that uses blood water as an endogenous tracer.

Methods—Bilateral forebrain ischemia of 4 minutes’ duration was induced in gerbils (n = 8). A modified version of FAIR with improved time efficiency was used to provide CBF maps with a time resolution of 2.8 minutes after recirculation had been initiated. Quantitative diffusion imaging was also performed at intervals during the reperfusion period.

Results—On initiating recirculation after the transient period of ischemia, the FAIR measurements demonstrated either a symmetrical, bilateral pattern of flow impairment (n = 4) or an immediate side-to-side difference that became apparent with respect to the cerebral hemispheres in the imaged slice (n = 4). The flow in each hemisphere displayed a pattern of recovery close to the preocclusion level or, alternatively, returned to a lower level before displaying a delayed hypoperfusion and a subsequent slow recovery. The diffusion measurements during this latter response suggested the development of cell swelling during the reperfusion phase in the striatum.

Conclusions—The CBF during the reperfusion period was monitored with a high time resolution, noninvasive method. This study demonstrates the utility of MRI techniques in following blood flow changes and their pathophysiological consequences. (Stroke. 1999;30:1263-1270.)

Key Words: animal models ■ cerebral ischemia, transient ■ magnetic resonance imaging ■ perfusion ■ reperfusion

After a stroke, reperfusion may occur spontaneously or via an interventional technique, such as thrombolysis. Animal studies of reperfusion after global ischemia have demonstrated that an impaired level of cerebral blood flow (CBF) may develop on recirculation.1 In models of focal ischemia, a varied range of flow levels have been observed on reperfusion.2 A few studies have suggested that a period of compromised blood flow after recirculation may not be pathogenic.3,4 Clinical reports of the pathogenesis of CBF impairment after reperfusion are limited,5,6 and this may reflect the time delay before most patients can be scanned. Because the use of thrombolytic therapy for the treatment of stroke is becoming more widespread, it is important to be able to understand any detrimental effects of recirculation. An aim of this study was to use the MRI techniques of perfusion and diffusion imaging to gain further insight into postreperfusion vascular and tissue status.

Two major categories of defects in circulation that follow a period of transient global ischemia have been characterized over the past 30 years. The first of these is known as the “no-reflow phenomenon,”7 in which areas of the brain fail to reperfuse. The alternative pattern is that of hyperemia followed by hypoperfusion.8

Many studies have been carried out that have aimed to characterize and follow the posts ischemic disturbances in circulation that occur after a period of transient global ischemia. An ideal study of posts ischemic reperfusion would track the time course of these changes in a single animal and would offer good regional differentiation, because a heterogeneous reflow might be expected. Previous investigations have used methods of perfusion quantification such as autoradiography using [14C]-iodoantipyrine,9 [3H]-nicotine,10 or [14C]-butanol11; hydrogen clearance12; xenon clearance13; and the technique of laser-Doppler flowmetry.14 All of these methods suffer from limitations in their application to such studies. For example, hydrogen clearance relies on the invasive placement of electrodes and provides measurements that are localized to the cortical volume in their immediate vicinity. The technique of xenon clearance is limited in spatial resolution and is susceptible to errors due to partial volume effects and high

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volumetric flow rates. Autoradiography, although displaying good spatial resolution, is invasive and does not permit longitudinal observations in a single animal. We have therefore implemented a technique for perfusion quantification that offers the relatively high spatial and temporal resolution of MRI.

The principal use of MRI is to obtain structural images of brain tissue. Recently, flow-sensitive implementations of the technique have been reported that allow quantitative maps of cerebral perfusion to be obtained. These techniques utilize the magnetic labeling of the water spins of inflowing blood or static tissue in the image voxel by the saturation or inversion of one with respect to the other. The flow-sensitive alternating inversion recovery (FAIR) technique was implemented in this study. Perfusion MRI was combined in this study with diffusion imaging, which provides measurements of the apparent diffusion coefficient (ADC) of tissue water. A reduction of the ADC is believed to be consistent with the development of cellular energy failure and the resultant cell swelling.

The purpose of this study was to use a combination of quantitative perfusion and diffusion imaging in an investigation of postischemic recirculation. A modification of the FAIR method was implemented that enabled a time-course study of cerebral perfusion with improved time efficiency. These techniques were used in an investigation of the reperfusion deficits that occur following a transient period of total forebrain ischemia in a gerbil model.

Materials and Methods
Adult Mongolian gerbils (n=8; weight, 60 to 70 g; B&K Universal Ltd, Hull, UK) were used in this study. The animals were anesthetized with 2% halothane. Anesthesia was subsequently maintained with a mixture of 1.5% halothane and 0.4 L/min of oxygen that was supplied via a nose cone, and the gerbils were allowed to breathe spontaneously throughout the study. The common carotid arteries supplied via a nose cone, and the gerbils were allowed to breathe spontaneously throughout the study.

CBF quantification in this situation, a series of global (half-passage adiabatic) saturation pulses were applied simultaneously along each gradient direction to eliminate signal contamination from fast-moving arterial spins. FAIR was implemented with a spin-echo echo-planar imaging sequence (TE=35 ms; imaging slice thickness, 2.3 mm; inversion slice thickness, 6 mm; image matrix size, 64×128; field of view, 27.5×55 mm). Images were obtained in the coronal plane. Slice-selective and non–slice-selective acquisitions were interleaved.

During the control phase of the experiment, a slice-selective inversion recovery set was acquired (recovery time, τ=6500 ms) at 7 inversion times (TI) (200 to 2500 ms). For 20 averages, this procedure lasted 22 minutes. The data were fitted for the parameters T1, T1app, α0, and M0 using the standard inversion recovery relationship (where α0 is the degree of inversion and M0 represents the equilibrium magnetization per unit mass of the tissue). These parameters are required for the quantification of perfusion.

For the time-course measurements of CBF, a reduced repetition time between the averaged acquisitions was used for rapid CBF mapping (TI=1300 ms, τ=1500 ms). To preserve the accuracy of CBF quantification in this situation, a series of global (half-passage adiabatic) saturation pulses were applied at the start of each recovery period. Previous experimentation had confirmed that perfusion values obtained with this implementation of FAIR were in excellent agreement with flows obtained with the more time-consuming biexponential fitting procedure. During the control period and acute phases of the experiment (occlusion and approximately 1 hour after reperfusion), 30 interleaved averages for each pair of selective and nonselective images provided a time resolution for CBF measurements of 2.8 minutes. During the later period of reperfusion, the number of averages was increased in order to increase the signal-to-noise ratio of the images with a 10-minute time resolution. The control values of M0 and α0 and the longitudinal tissue relaxation time, T1, were used to obtain perfusion measurements directly from each subtraction image. Previous studies in which inversion recovery data sets were acquired at approximately half-hour intervals during the reperfusion period had demonstrated that the values of these parameters did not change during this phase at our field strength (n=2; data not shown). A value for the T1 of blood, T1b, of 1500 ms was obtained by extrapolation of published data acquired at other field strengths.

The transmitter coil inflow time, Δ, must be taken into account to determine CBF in the situation of an RF coil that does not provide complete coverage of the body. This defines the time subsequent to the application of the inversion pulse, after which blood spins will begin to enter the imaging slice from outside the RF transmitter coil. A value for this time was experimentally determined in a control experiment by obtaining values for the magnetisation difference while varying the recovery time. Comparison of the data with...
the theoretical relationship of these 2 variables enabled a value for Δ of 1800 ms to be estimated.22

Diffusion MRI
Measurements of the trace of the diffusion tensor, denoted trace(D), were obtained in 5 of the animals during the control period and at approximately half-hour intervals during the postreperfusion phase. The trace(D) is a rotationally invariant measure of the diffusion tensor, and a number of sequences have been devised that enable its acquisition by a single scan. The single-shot trace(D)-weighted echo-planar imaging sequence25 used in this study was implemented with the following parameters: TE 5 110 ms; TR 5 1000 ms; number of averages 5 30; b values 5 0; and 1187 s/mm².

Image and Data Analysis
Perfusion values were calculated pixelwise by importing the data into IDL (Floating Points Systems). The relevant reduced repetition time equation for the magnetisation difference provides a nonlinear expression for the CBF. An iterative root-finding routine was used to solve the equation for flow, thus providing a CBF map from each subtraction image (selective–nonselective). Four regions of interest were drawn on these perfusion maps in the left and right hemispheres of the slice, in both the cortex and the corpus striatum. All values are presented as mean±SD. Statistical analyses of the factorial (region×group) within-subjects data were performed by mixed-model regression analysis using appropriate random effects models. A value of P<0.05 was considered to indicate statistical significance.

To characterize certain features of the flow response during the acute phase of the reperfusion, a suitable random-coefficients model was fitted to the first hour of the reperfusion data. Maximum likelihood nonlinear mixed model regression was performed with use of an SAS macro (NLINMIX, supplied by R. Wolfinger, SAS Institute, Cary, NC). A detailed models comparison study was not performed. Instead, an exponentially damped polynomial model was adopted of the form

\[ f(t) = \alpha + (\beta_0 + \beta_1 t) \exp(-\lambda t) \]

where \( f \) represents the flow; \( t \) is the time since the occlusion; and \( \alpha, \beta_0, \beta_1, \) and \( \lambda \) are random coefficients. This model was selected since it is economical in the number of coefficients and provides an adequate description of the zero-time and asymptotic behavior .

Results
CBF measurements were obtained with the reduced repetition time implementation of FAIR. Equation A11 in Reference 22 was used to obtain CBF maps from each subtraction image, because the conditions \( \tau<\Delta \) and \( TI<\Delta \) that define this equation were satisfied in our experiment. The mean preocclusion flow measurements were, in the right cerebral hemisphere, 155±27 mL/100 g/min in the cortex and 132±26 mL/100 g/min in the striatum; and in the left hemisphere, 161±25 mL/100 g/min in the cortex and 129±26 mL/100 g/min in the striatum (n=8).

Representative perfusion maps are displayed in Figure 1. After recirculation had been initiated, a marked variability in the response to recirculation became obviously apparent on visual inspection. Based on these observations, the responses observed in the cerebral hemispheres of each animal were
assigned to 2 groups (groups A and B). The group response reflected the characterization of the reperfusion time course in each hemisphere. In each of the 8 animals, at least 1 cerebral hemisphere displayed an early transient recovery of CBF that was followed by a period of hypoperfusion. Hemispheres that showed this response were assigned to group B. In 4 of the 8 animals, a side-to-side difference became apparent and the group B hemisphere was accompanied by the observation in the contralateral hemisphere of a permanent immediate renormalization of the flow (Figure 1, panel a); these hemispheres were assigned to group A. Each of the 4 animals thereby provided 1 group A and 1 group B hemisphere. The other 4 animals displayed a bilateral group B response (Figure 1, panel b). The complete data set of 8 animals, therefore, provided 4 group A hemispheres and 12 group B hemispheres. Figure 2 shows the time-course of the CBF values for the data sets in each of the 2 groups.

Table 1 details the control and occlusion flows for group A and group B data. Mixed model regression analysis of the pre-occlusion data showed a significant regional (cortex versus striatum) difference (P=0.01) while the difference between the 2 groups (group A versus group B) was not significant. There was no significant regional or group differences in the flow values measured during the occlusion phase.

**Time-Course CBF Data: Group A (Immediate Normalization)**

This response of immediate normalization of the flow never occurred in both cerebral hemispheres of the same animal. On recirculation, the blood flow recovered to a value close to the pre-occlusion level. In order to characterize the initial recovery and the subsequent flow response, an exponentially damped polynomial was fitted to the first hour of the postreperfusion data (Equation 2). To test for regional differences, 2 such models were compared, one of which is a full model and consists of a separate set of coefficients for the cortical and striatal regions; the second model is a reduced model and assumes a common set of coefficients for the 2 regions. The difference between the models was not significant (log-likelihood ratio, \( \chi^2 \)); the reduced, region independent model was therefore adopted. The calculated mean time-dependent behavior is shown in Figure 3. The level of the initial recovery on reperfusion was provided by the peak of the fitted model. A value of 146 mL/100 g/min at 11.7 minutes after reperfusion (ie, at 15.7 minutes after occlusion) was thereby obtained. This maximal flow is 8% lower and 12% higher than the preocclusion levels in the cortex and striatum, respectively. At the end of the 1-hour period, the CBF reached an asymptotic level of 126 mL/100 g/min.

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**Table 1: Mean Preocclusion and Occlusion Flows in the Cortex and Striatum**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
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<tbody>
<tr>
<td></td>
<td>Cortex</td>
<td>Striatum</td>
</tr>
<tr>
<td>Preocclusion</td>
<td>152±21</td>
<td>120±20</td>
</tr>
<tr>
<td>Occlusion</td>
<td>6±13</td>
<td>12±23</td>
</tr>
</tbody>
</table>

Cortical and striatal perfusion values are given in milliliters per 100 grams per minute (mean±SD). Values are shown for group A (n=4 independent hemispheres) and group B data (n=8 independent hemispheres).
model was chosen to provide an adequate description of the data. The maximal level of the initial recovery on reperfusion was provided by the peak of the fitted model (Figure 3). A value of 80 mL/100 g/min at 4.9 minutes after reperfusion (ie, 8.9 minutes after occlusion) was thereby obtained. This flow is 49% lower and 38% lower than the mean preocclusion levels in the cortex and striatum respectively and, therefore, the mean response is not suggestive of a period of true reactive hyperemia. The calculated mean flow subsequently dropped to an asymptotic level during this 60-minute period of 13 mL/100 g/min.

At approximately 60 minutes after reperfusion, the blood flow began to recover slowly. The mean slope of the recovery obtained by linear regression of data collected from 1 hour after reperfusion until the end of the experiment was significantly different from zero with values of 9.8±2.6 (mL/100 g/min)/h and 9.4±4.6 (mL/100 g/min)/h in the cortical and the striatal regions (P≤0.0002). By 3 hours after reperfusion, the CBF had reached a level of 35±9 mL/100 g/min and 37±14 mL/100 g/min in the cortex and striatum, respectively.

Discussion
The principal findings of this investigation were the demonstration of the benefits of using rapid FAIR imaging in a time-course

Time-Course CBF Data: Group B (Recovery Followed by Hypoperfusion)
This response occurred in 50% of the animals as a unilateral response and in the other animals as a bilateral response; in the latter situation, regional data were averaged over the 2 cerebral hemispheres. On initiation of recirculation, the blood flow recovered and, at approximately 5 minutes of reflow, reached a maximal level. A period of hypoperfusion then followed. An exponentially damped polynomial was fitted to the first hour of the postreperfusion data (Equation 2). The likelihood ratio test comparing a full, region-dependent coefficients model with the restricted common coefficients model was not significant. Therefore, a common coefficients
The mean global preocclusion CBF value obtained with FAIR was $144 \pm 21$ mL/100 g/min. This control flow is in general agreement with figures quoted in previous work with gerbil models. On reperfusion, the initial return, in all cases, of the CBF to values close to the preocclusion level precludes the presence of a general no-reflow phenomenon. There was no indication of any immediate focal impairments in CBF on reperfusion even in the striatum, which has been shown to be one of the subcortical regions that is susceptible to no-reflow.

The immediate and permanent recovery of perfusion that was observed unilaterally in a number of animals (group A response) was unexpected. On 4 occasions, at the conclusion of the experiment, the animal was removed from the magnet and the snares were examined. The pulsation of the arterial flow proximal and distal to the snare was checked for consistency. At the end of every study, a postmortem examination was carried out to verify that the snare was fully open and that the external diameter of sections of the carotid arteries proximal and distal to the snare was constant. No evidence of an unsuccessful occlusion or reperfusion was thereby found. It has been noted that there is a variability in the number and the size of the small communicating blood vessels between the vertebralbasilar and the carotid circulation in the gerbil. The degree of collateral circulation that originates from the unoccluded vertebral supply might therefore be the cause of the observed side-to-side asymmetry on reperfusion. Previous studies have noted the side-to-side variations in the vasculature of the circle of Willis in gerbils.

The postischemic impairment in reflow that was observed in this study (group B response) followed the pattern of an initial recovery of flow, a delayed period of hypoperfusion, and then a slow recovery in the direction of the preocclusion level. The diffusion trace(D) measurements after reperfusion suggested a decreasing trend with time. However, further work is required to confirm this diffusion response with more animals. The diffusion coefficient and the longitudinal relaxation time, $T_1$, are sensitive to temperature but the nonselective, $T_1$-weighted FAIR data provided no evidence of temperature-related changes. The decreasing trend in the trace(D) values may reflect a process of progressive cell swelling during the phase of hypoperfusion. This trend was especially apparent in the striatal regions, which suggests a regional difference in susceptibility to injury. A similar regional pattern was observed in a recent study of hypoxia-ischemia in the rat. The magnitude of the decline in the trace(D) values observed in the striatum was substantially smaller than the change typically observed during acute ischemia. This observation is consistent with the CBF remaining close to the flow threshold for diffusion changes. Our laboratory has demonstrated the existence of a flow threshold of approximately 20 mL/100 g/min in the gerbil. These findings suggest that further work must be performed to elucidate whether the regional ADC change during the hypoperfusion period contributes to any long-term, deleterious effects of recirculation. In previous studies of the ADC changes that occur after reperfusion, several distinct responses have been observed, including immediate normalization and a delayed decline related to secondary energy failure.

The CBF during the period of delayed hypoperfusion (group B response) dropped to an unexpectedly low level.
based on previous similar studies. It has been suggested that, paradoxically, shorter periods of ischemia result in a more pronounced degree of postischemic hyperperfusion. For example, 30 minutes of total forebrain ischemia in the gerbil resulted in a delayed drop in flow that represented approximately 40% of the control level (hydrogen clearance technique for CBF measurement), while after 5 minutes of ischemia in a similar gerbil model, the CBF fell to a value that was approximately 15% of the preocclusion flow (autoradiography technique). This relationship between the duration of the period of ischemia and the severity of hyperperfusion has however, not been verified in a single study.

It is also probable that the flow is being underestimated by the FAIR measurement during the period of compromised perfusion. The first postreperfusion measurement of the diffusion coefficient displayed no significant change, and this indicates that the level of flow is above the threshold for diffusion changes. The underlying cause of the inaccuracy in the flow measurement is the effect of the reduction in the blood flow on the validity of certain assumptions of the FAIR model. The reduced blood flow will increase the significance of the transit time, during which blood originating from within the inversion slab will move into the imaging slice. This duration exists as a result of the increased width of the selective inversion slice relative to the imaging slice, which is necessary to eliminate effects from imperfect edges of the inversion and the imaging pulse profiles. The effects of δ are not normally considered when analyzing FAIR data, because this time is expected to be insignificant for normal levels of flow. However, the impairment of flow during the period of hypoperfusion will increase the transit and inflow times. If these increased times are not taken into account in the FAIR quantification model, the technique will provide underestimated flow values. The sensitivity of the flow measurements to a changing transit time or inflow times can be theoretically determined. For example, with our combination of parameters and for a real flow of 20 mL/100 g/min, the transit and inflow times would both need to rise by approximately 300 ms to induce an underestimation in the measured flow value of 5 mL/100 g/min. Improved inversion and imaging slice profiles are being investigated in our laboratory with a view to reducing the effect of the transit time on the quantification.

In conclusion, we have demonstrated the use of noninvasive FAIR perfusion imaging in following regional CBF changes with a high time resolution in a longitudinal study of reperfusion. On initiation of recirculation after 4 minutes of total forebrain ischemia, the blood flow in the cerebral hemispheres either returned to the preocclusion level or displayed a pattern of initial recovery followed by hypoperfusion. The combined measurements of CBF and the diffusion trace suggest a period during the reperfusion phase when intervention may be necessary to ameliorate recovery. The ability to follow and characterize a time course of events with quantitative mapping of cerebral perfusion has obvious potential in the investigation of stroke.

References


Measurement of cerebral blood flow noninvasively has been an elusive goal since Kety and Schmidt1 first measured blood flow. In this article the authors describe the flow-sensitive alternating inversion recovery (FAIR) method that spin-labels flowing blood and follows it through a tissue region.2 With the aid of computer algorithms, they have shortened the acquisition times, making flow maps in several minutes. Technical requirements are still daunting. Echo-planar instruments and specially designed pulse sequences are required. However, as with other MR methods, clinical applications should follow.

Diffusion-weighted imaging (DWI) shows the state of water in tissue.3 When cells are swollen, reducing the extracellular space, the diffusion coefficient falls. Because extracellular space, the diffusion coefficient falls. Because

Using the well-established gerbil model of ischemia, they occluded the carotids for 4 minutes within the magnet.5 Several patterns of flow occurred after the release of the occlusions. Some animals had no change in flow, while others showed hyperemia followed by decreased flow ("no-reflow").6 Variability was seen in the flow patterns between hemispheres in the same animal. To overcome the variability, hemispheres were grouped according to flow rather than by animal. The data showed that the poorly perfused hemispheres had a slight fall in diffusion coefficients rather than the expected significant change. They suggest that the lack of correlation is due to an underestimation of true values when flow is impaired. The strength of this report lies in the methodology rather than in new physiological data. In fact, more work will be needed to clarify the potential errors in the measurements.

Characterization of the state of the tissue after a stroke is critical for the use of thrombolytics and fibrinolytics that have entered clinical use. Selecting patients that have potentially salvageable brain tissue and do not have an increased risk for hemorrhage is a major challenge.7,8 Quantification of blood flow, measurements of diffusion constants, and biochemical measurements with spectroscopy provide a 3-dimensional view.9 Speeding up the acquisition of MR data will add a time dimension. This report adds another step in the rapid advance in MR methods. It should provide information about tissue viability that will guide therapies.

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References
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