Middle Cerebral Artery Occlusion in Rats Studied by Magnetic Resonance Imaging

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Ischemia due to middle cerebral artery occlusion was studied in 29 rats from 1 to 24 hours after occlusion using magnetic resonance imaging. Images were made before and after the injection of a superparamagnetic iron oxide compound, AMI-25. Subtraction images demonstrated the region of perfusion deficit as early as 1 hour after occlusion, earlier than conventional T2-weighted images. The area of altered perfusion detected by this technique (subtraction imaging after AMI-25 administration) correlated with that demonstrated by iodoantipyrine autoradiography. Since this magnetic resonance technique can be used to serially estimate the location and size of the ischemic area, the technique can be an important adjunct to metabolic studies of focal ischemia using magnetic resonance spectroscopy. The technique may have clinical applications as well. (Stroke 1989;20:1032-1036)

A major problem in the study of animal models of stroke is variability in the size and location of the ischemic lesions. This problem complicates experiments requiring serial in vivo measurements of the metabolic status of the involved brain. Since magnetic resonance spectroscopy can serially define these metabolic changes,1-3 the study of focal ischemia would be aided by magnetic resonance imaging (MRI) methods that could also serially define the volume of ischemic brain with precision yet without elaborate or time-consuming techniques. Although this problem obviously does not concern studies of global ischemia, it is important to develop magnetic resonance methodology to study focal lesions that will complement studies of global ischemia since it is focal lesions that are most often encountered clinically. We are addressing this issue using MRI with contrast agents in a model of focal ischemia, middle cerebral artery occlusion in rats. Paramagnetic compounds enhance proton relaxation times, thereby altering the intensity of the images. Paramagnetic compounds that are retained in the intravascular space for adequate lengths of time can thus be used to image volumes of altered perfusion. We have previously demonstrated hemispheric differences in perfusion in a rat three-vessel occlusion model using gadolinium–diethylenetriaminepentaacetic acid complexed to albumin.4 In our current experiments, we employed a superparamagnetic iron oxide formulation, AMI-25 (Advanced Magnetics Inc., Cambridge, Massachusetts) more potent than gadolinium. AMI-25 has been used to image the liver5 but has not been used to study the brain. The effects of AMI-25 are readily visualized with T2-weighted spin–echo imaging. Using middle cerebral artery occlusion in rats and intravenous injection of AMI-25, we can detect focal ischemia early.

Materials and Methods

Twenty-nine adult Sprague-Dawley rats (200–300 g) that had been allowed free access to food and water were anesthetized with a 90:10 mg/kg i.m. ketamine: xylazine mixture. Arterial and venous access via femoral catheters was established, and blood pressure and arterial blood gases were monitored. Core body temperature was maintained using a circulating water bath (K-module, Hamilton, Cincinnati, Ohio). Ipsilateral common carotid and middle cerebral arteries were occluded permanently according to the method described by Brint et al.6 After shaving and providone-iodine preparation of the ventral neck and the region of the right zygoma, the right common carotid artery was dissected via a midline cervical incision and ligated. The rat was...
then placed in a stereotactic headholder with the right side of the head superior. A 1-cm incision was made midway between the lateral canthus and the external auditory canal. The temporal muscle was incised and retracted. Using continuous saline irrigation and magnified vision, a 2-mm craniec-tomy was made just rostral to the zygomatic–squamosal suture. The dura was incised with a 25-gauge needle. The tip of a 30-gauge stainless steel wire, fashioned with a small right angle, mounted on a micromanipulator was then placed under the middle cerebral artery at a point approxi-mately 1–2 mm distal to the entorhinal fissure, and the artery was elevated away from the under-lying brain. The artery was then coagulated with a 20-mA current through the wire and an adjacent scalp electrode and was cut with microscissors.

MRI was done using a 4.7-T CSI system (General Electric, Milwaukee, Wisconsin). All images were made using a 1.3-in. homemade birdcage coil tuned to 200.1 MHz for proton imaging. Multislice $T_2$-weighted spin–echo transverse (coronal) images of resonance time 2,500 msec and echo time 60 msec were acquired with a 10×10-cm field of view, 3-mm slice thickness, and 128 or 256 phase-encoded steps. The centers of neighboring slices were separated by 4 mm. Images were obtained in 29 rats before and after intravenous injection of iron oxide as AMI-25 (70–100 μl, 2–4 mg elemental iron/kg body wt). Five rats were imaged at <3 hours, 16 rats at >3 hours, and eight rats were imaged twice. In other experiments, changes in intensity before and after injection of AMI-25 measured in anesthetized but otherwise normal rats had ranged from 40% in gray matter structures to 20% in the corpus callosum; side-to-side differences were virtually 0 (unpub-lished data). Subtraction images were obtained by matrix subtraction of postinjection from preinjec-tion images. Images were obtained as early as 1 and as late as 24 hours after occlusion. Lesions were detected by visual inspection for the typical appear-ance of infarction (high-intensity lesion) on a preinjection image or for decreased perfusion (low-intensity area) on a subtraction image.

Cerebral blood flow was quantified using the autoradiographic iodio[14C]antipyrine ([14C]IAP)
method\textsuperscript{7} in seven rats that were also investigated with MRI. In these seven rats, \[^{14}\text{C}\]IAP was injected 15 minutes after the injection of AMI-25. During the approximately 1-minute infusion of \[^{14}\text{C}\]IAP, arterial blood samples were collected from the freeflowing arterial catheters onto filter paper disks placed in small plastic beakers. Sampling time was corrected taking into consideration the dead space of the cannula. At approximately 1 minute, the rats were decapitated and the brains were quickly (within 40–50 seconds) removed and frozen in isopentane at $-45^\circ\text{C}$. The weight of the blood was calculated as the difference in the weight of the beakers before and after sampling. The filter paper disks were transferred to scintillation vials, and the amount of carbon-14 per sample was calculated by liquid scintillation counting (LKB Instruments Inc., Gaithersburg, Maryland). The concentration of carbon-14 per unit volume of blood was calculated from the measured amount of carbon-14 and the weight of the blood sample, assuming a specific gravity of 1.05 g/ml for blood.

The brains were cut into 20-$\mu$m sections in a cryostat (Hacker Instruments, Fairfield, New Jersey) at $-22^\circ\text{C}$. The sections were thaw-mounted on glass coverslips, dried on a hot plate at 60$^\circ\text{C}$, and autoradiographed with type OM1 x-ray film (Eastman Kodak Co., Rochester, New York) along with a set of calibrated \[^{14}\text{C}\]methylmethacrylate plastic standards. Local cerebral tissue concentrations of \[^{14}\text{C}\]IAP were determined from the optical densities of the brain structure in the autoradiographs and a calibration curve derived from the relation between optical density and a known concentration of the standard using an image analysis system described by Lear et al\textsuperscript{8}; local cerebral blood flow was calculated according to the operational equation of the method.\textsuperscript{7} Comparable regions on autoradiographs and MRI images were visually selected by a trained observer. The area of hypoperfusion was outlined, and the ratio of the number of pixels in this region and the number of pixels in the entire slice was computed by the observer and independently by an investigator that was blinded as to matching the autoradiographs and MRI images.

The brains of 22 rats used for MRI only were fixed in formalin, cut into 20-$\mu$m coronal sections, and stained with hematoxylin and eosin for histologic identification of the infarct. In the cerebral blood flow studies, autoradiographed sections from seven rats were stained with hematoxylin and eosin for histologic identification of the infarct.

**Results**

Figure 1, top shows representative preinjection and postinjection MRI images from a rat taken 24 hours after middle cerebral artery occlusion. The subtraction image and a stained coronal section from the same level in the same rat are shown in Figure 1, bottom. At 24 hours, as expected, MRI showed an increased intensity in the area of the perfusion deficit. This area was even more pronounced in the subtraction image, and an infarct was confirmed by histology. \(T_2\)-weighted images taken 1 hour after occlusion are shown for another rat in Figure 2, top. Whereas images made before the injection of AMI-25 do not show a perfusion deficit, the subtraction image demonstrates an area of decreased perfusion in the middle cerebral artery territory when no gross lesion could be detected by histologic examination of the stained sections (Figure 2, bottom). We compared our ability to detect...
TABLE 1. Lesion Detection by T2-Weighted Magnetic Resonance Images Before and After Injection of AMI-25 Into Rats With Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Time after occlusion</th>
<th>Before AMI-25</th>
<th>After AMI-25</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 hr (n=13)</td>
<td>No. 0</td>
<td>13 100%</td>
</tr>
<tr>
<td>≥3 hr (n=24)</td>
<td>19 79%</td>
<td>24 100%</td>
</tr>
</tbody>
</table>

perfusion deficits before and after injection of AMI-25 for observations made early (<3 hours) and later (≥3 hours) after occlusion (Table 1). Early preinjection images did not show a deficit in any of 13 rats studied, whereas after injection deficits were seen in all 13 rats. In addition, in late preinjection images, deficit was detected in 79% of 24 rats; injection of AMI-25 increased our ability to detect a deficit to 100%. At 24 hours all of these 24 rats had infarcts confirmed histologically.

Seven rats were studied by both MRI and autoradiography to compare the location and size of the perfusion deficits as estimated by these two techniques. Blood pressure and Pao2 and Paco2 did not change during both studies and during the brief interval between them. Figure 3 shows a representative subtraction image taken 1 hour after occlusion; the image has been color-coded for easier comparison with the autoradiograph made at the same level in the same rat. Autoradiography shows that the border of the perfusion deficit is sharp; that is, there is no large gradient zone around the area of perfusion deficit. The measured area of the deficit was similar by the two techniques. The areas were compared for the seven rats, and the correlation is shown in Figure 4. While the size of the perfusion deficit varied among rats, there was a good correlation between areas estimated by the two techniques ($r=0.8$).

FIGURE 4. Area of perfusion deficit as fraction of total area in rat brain after middle cerebral artery occlusion estimated by T2-weighted magnetic resonance imaging with AMI-25 and by iodo[14C]antipyrine autoradiography.

Discussion

AMI-25 has a half-life of approximately 20 minutes and therefore seems suitable for use as a contrast agent in assessing the size and location of perfusion deficits by serial MRI. Our data indicate that perfusion deficits can be visualized ≤3 hours after occlusion, before there is demonstrable gross pathology and before changes can be detected reliably by standard proton MRI techniques. Further, we demonstrate that perfusion deficits as early as 1 hour after occlusion detected using AMI-25 are similar in size and location to those detected by a more conventional method but one that requires killing the animal, regional cerebral blood flow assessment using [14C]IAP autoradiography. We believe that this kind of in vivo information about the size and location of perfusion deficits will aid in experiments measuring metabolic changes by mag-
netic resonance spectroscopy, either by more accurate placement of surface coils and better estimation of the relative volume of perturbed tissue in their fields of view or by spectral measures using phase encoding in which relative proportions of normally perfused and perturbed tissue can be estimated for each slice individually.

The threshold of blood perfusion detectable using AMI-25 is not yet known. However, in a model such as ours, the territory involved in ischemia is discrete and readily measurable. AMI-25 may also be applicable for the study of ischemia and infarction in patients since it has been given intravenously to patients for imaging the liver, although in lower doses.5

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References

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