Microsphere-Induced Embolic Stroke: An MRI Study

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Despite the many studies of the middle cerebral artery occlusion (MCAO) model, efficient therapy for stroke is still lacking, emphasizing the need for further development and characterization of experimental stroke models. In the present study, the rather unexplored multifocal microsphere-induced stroke model in rats was characterized by multiparametric MRI. We induced microembolic infarction in a group of Sprague-Dawley rats by injecting a dose of about 1000 50-μm polyethylene microspheres intracranially from the external carotid artery. Diffusion-, perfusion-, and T2*-weighted MRI were used to evaluate the infarct development during and following the first 3 hr after microsphere injection (N = 20). The animals were also imaged at 12-hr (N = 8), 24-hr (N = 17), and 48-hr (N = 5) time points. After the final imaging time point, the brains were removed and sectioned into 2-mm-thick slices, and infarct volumes were measured by 2,3,4-triphenyltetrazolium chloride (TTC) staining. From calculated apparent diffusion coefficient (ADC) maps, a volume of reduced ADC appeared 0.5–1.0 hr postinjection, and by the 3-hr time point the volume of ADC reduction had increased to a size of 5% ± 1% (mean ± SEM) of the brain hemisphere. The lesion volume increased significantly (P < 0.01) to 16% ± 2% of the hemisphere volume at the 12-hr time point, while at 24 hr the lesion (15% ± 2% of the hemisphere) was also significantly larger (P < 0.001) than at 3 hr. The perfusion deficit resulting from the microsphere injection was immediate, going from a cerebral blood flow index (CBF) of 74% ± 3% at the time of microsphere injection to 68% ± 2% of the contralateral mean at 3 hr (P < 0.05), to 55% ± 4% of the contralateral values at 12 hr (P < 0.05), and to 57% ± 2% of the contralateral mean at 24 hr (P < 0.001). The lesion development in the microsphere-induced stroke model was found to be slower than in the MCAO model, and continued up to the 24–48-hr time point. Magn Reson Med 51:1232–1238, 2004. © 2004 Wiley-Liss, Inc.

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Stroke, or cerebral ischemia, is the third largest cause of death and the prime cause of permanent disability in western countries. In the United States alone, stroke affects over 500000 people each year, of which roughly 30% die and another 20–30% become severely and permanently disabled (1). The constant efforts to develop, characterize, and apply experimental models of stroke for the screening of new neuroprotective drugs are understandable, given the devastating outcomes of stroke (1,2). In the last two decades, many experimental models for stroke have been introduced, and MRI has been found to be an efficient tool for their characterization (3).

Numerous animal models of stroke have been studied by MRI (3). The most widely used models are the middle cerebral artery occlusion (MCAO) model and its variants (4,5). The MCAO model is considered to be reproducible, and it results in a relatively large and homogeneous lesion. This makes its characterization by different imaging techniques in general, and by MRI in particular, relatively easy (3,6,7). These models have proven useful for the successful preclinical evaluation of neuroprotective agents; however, subsequent clinical evaluations of these same compounds have been less rewarding (8). One of the reasons for the clinical failure of these agents may be the fact that animal-model studies do not entirely mimic the clinical features of stroke. For example, the MCAO model is studied preferentially because of its consistency in producing homogeneous lesions of predictable size, although it is well known that lesions in clinical stroke display variability in cerebral blood flow and infract volume (9), as well as in the morphology and localization of the lesions (10). Several embolic models have been proposed to better simulate the clinical situation, some of which utilize multiple blood clots (11) or a single blood clot (12) to induce stroke. These models basically emulate the MCAO model with a clot or an accumulation of clots. Other models that use multiple blood clots (13) are difficult to perform reproducibly. These models are especially useful in the study of thrombolytic therapy, but are susceptible to spontaneous lysis of the emboli. Therefore, there is a constant need for the development and characterization of new experimental models of stroke that better emulate the clinical situation.

The microsphere-induced embolic stroke model (14) is a multifocal model of permanent occlusion. The size and the dose of the injected microspheres can be used to regulate the extent and severity of the resulting lesions (15). Although it is a rather unexplored model of stroke, several studies have examined the behavioral (16), biochemical (17), neurological, and histological (18,19) changes that occur in this model. Most of these studies have concentrated on the long-term effects of microsphere-induced embolism (16), but some of the biochemically-oriented studies have studied the changes that occur within the first hours after the insult (17).

In experimental stroke, T2*-diffusion-, and perfusion-weighted MRI are widely used to characterize different stroke models (3,6,7,12,13). However, MRI was not utilized in the study of microsphere-induced embolic stroke until recently (20). This recent study employed T2* and...
We prepared a suspension of 50-μm-diameter microspheres by adding 10.4 mg of microspheres to 30 mL of normal saline, to yield a stock solution of about 1000 microspheres per 0.2 mL solution. After the microspheres were mixed with saline, the microsphere suspension was agitated with a vortex mixer (Labcraft Super Mixer II 250-490; Lab-Line Instruments Inc., Melrose Park, IL) and placed in an ultrasonic bath (Ultrasonic Cleaner ME 2.1; Mettler Electronics Corp., Anaheim, CA) for 20 min. The suspension was then stored at 4°C until just prior to use. In preparation for injection, the microsphere suspension was placed in the ultrasonic bath for 40 min and agitated again with a vortex mixer. Then, 0.2 mL of the suspension was withdrawn into a 1-mL syringe and immediately used as detailed below.

Anesthesia

For the preliminary surgery, anesthesia was induced by an intraperitoneal injection of chloral hydrate (400 mg/Kg body weight). During the MRI experiments and the in-bore occlusion procedure, anesthesia was maintained by 1.0% isoflurane delivered in air at 1.0 L/min.

Animal Preparation

In the preliminary surgery stage, PE-50 tubing was inserted into the left femoral artery for continuous monitoring of arterial blood pressure (Parametron 7150 monitor; Roche Medial Electronics, Cranbury, IL) throughout the study, and to measure arterial pH, PaO₂, and PaCO₂ (Corning 178-pH blood gas analyzer; Corning, Inc., Mansfield, MA) at baseline, immediately after injection of the microsphere suspension, and 1 hr and 3 hr thereafter. Another PE-50 catheter was inserted into the inferior vena cava through the left femoral vein for the Gd(DTPA) (Magnevist®, Berlex Laboratories, Wayne, NJ) injections used for perfusion-weighted imaging (PWI). Body temperature was monitored with a rectal probe and maintained at 37.0°C with a thermostatically-controlled heating lamp (model 73ATD; YSI Inc., Yellow Spring, OH) during the surgery. The experimental procedure was performed on 20 animals, all of which were injected with a 1000-microsphere dose, as described by Bralet et al. (14), in the magnet. Briefly, the common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed through a midline incision in the neck. The pterygopalatine artery was ligated with a 5-0 surgical suture, and tubing was introduced into the ICA through an arteriotomy of the ECA and advanced approximately 15 mm. The proximal portions of the ECA and the pterygopalatine artery were ligated with 5-0 surgical sutures, and the proximal portion of the CCA was also ligated. The intra-arterial tubing was inserted through an arteriotomy of the CCA, 3 mm below the carotid bifurcation, and fixed by double ligation. Body temperature was continuously monitored with a rectal probe (T-type thermocouple; OMEGA Engineering Inc., Stanford, CA) and was maintained at 37°C ± 0.2°C by means of a thermostatically-regulated, heated airflow system. After preocclusion imaging was performed, embolization was achieved by injection of an aliquot of microsphere suspension and flushing with 0.2 mL saline.

Histology

After the final imaging time point, the rats were anesthetized with an intra-peritoneal injection of chloral hydrate (400 mg/Kg body weight) and decapitated. The brains were immediately removed and sectioned coronally into six contiguous 2-mm-thick slices. The brain slices were incubated for 30 min in a 2% solution of 2,3,4-triphenyltetrazolium chloride (TTC) at 37°C and fixed by immersion in a 10% buffered formalin solution. The brain slices were then photographed with a CCD camera (EDC-1000HR Computer Camera; ELECTRIM Corp., Princeton, NJ).

MRI Methods

The MRI experiments were performed with a GE CSI-II 2.0T/45 cm imaging spectrometer (GE NMR Instruments, Fremont, CA) operating at 85.56 MHz for 1H and equipped with ±20 G/cm self-shielding gradients. The methods used for the MRI were described previously (6). Briefly, diffusion-weighted, spin-echo, echo-planar imaging (DW-EPI) (22) was used to map the average apparent diffusion coefficient (ADC avg) of tissue water, which is equal to the trace of the apparent diffusion tensor (23,24), as previously described (25). We achieved a range in b-values by incrementing the gradient amplitude, g, from 2 to 18 G/cm, in 2-G/cm steps. Eight contiguous, axial, 2-mm-thick slices, centered on the optic chiasm, were acquired with TR = 5 s, TE = 74 ms, EPI acquisition time = 65 ms, NEX = 2, δ = 7 ms, and Δ = 35 ms. The six center slices of the diffusion data, corresponding to the position of the TTC slices, were used to calculate the lesion volume.

T₂*-weighted EPI was employed to perform dynamic contrast-enhanced PWI (26). Four contiguous, coronal 2-mm-thick slices, which correspond to the position of the four center diffusion slices, were acquired. A set of 40 images (TR = 900 ms, TE = 74 ms, EPI data acquisition time = 65 ms, NEX = 1) was obtained for each slice. A bolus injection of 0.25 ml of Gd(DTPA) (Magnevist®, Berlex Laboratories, Wayne, NJ) was administered following acquisition of the 15th image. The PWIs were processed as described previously (27). Only relative changes in cerebral blood volume (CBV) were evaluated, and thus knowl-
edge of the tissue contrast agent concentration and the arterial input function was not required. The relative cerebral blood volume (rCBV) was determined by numerical integration of the ΔR₂(t) vs. time curve. An estimate of the vascular transit time (VTT) was obtained from the first moment of the ΔR₂(t) vs. time curve, and was then used in conjunction with the value for rCBV to calculate an index of the cerebral blood flow (CBFi, = rCBV/VTT) based on the central volume principle, where CBFi was determined for each pixel.

A multislice, double spin-echo EPI sequence was used to map the T₂ of the brain. T₂ maps were constructed from nine T₂-weighted EPIs with a TR = 5 s, NEX = 4, and TEₙ values of 20–110 ms. The TE for the second echo was the same as the TE for the diffusion-tensor (TE₂ = 74 ms) and PWI sequences, to ensure that the diffusion, perfusion, and T₂ maps all contained the same EPI spatial distortions. Eight contiguous, 2-mm-thick coronal slices, centered about the optic chiasm, were acquired. An FOV of 25.6 mm × 25.6 mm, and 64 × 64 pixel resolution were used for all the images.

Acute ischemia was characterized (N = 20) by the acquisition of data for the ADCav, T₂, CBFi, maps preocclusion, at the injection time, and at 1, 2, and 3 hr thereafter. Diffusion data were also acquired at 30, 90, and 150 min postinjection. We had also planned to image 12 animals at 24 and 48 hr postinjection; however, only five of those animals survived up to the 48-hr imaging session. Eight additional animals were imaged at 24 and 24 hr postinjection; therefore, 17 animals were imaged at the 24-hr time point. It was not possible to image the same animals during the first 3 hr and again at 12, 24, and 48 hr, because of the risk of a high mortality rate.

Data Processing and Analysis

We calculated the lesion on the TTC image using image analysis software (BioScan OPTIMAS, Edmonds, WA), and calculated the lesion volume as the percent hemispheric lesion volume (% HLV) using the method reported by Li et al. (6). In the present study, the deviations in the ADC/T₂ vs. TTC lesion volume with respect to the x = y line were minimized (28). The sum of squared differences (SSD) between the ADC/T₂- and histology-derived lesion volumes was used as a goodness-of-fit criterion, and minimization of SSD yielded an ADC/T₂ estimate that gave the best fit to the model x = y line. Lesion volumes (reported as a percentage of the hemisphere) were determined by means of in-house-developed software written in IDL (Interactive Data Language; Research Systems Inc., Boulder, CO). Lesion pixels were distinguished from normal tissue, on a pixel-by-pixel basis, by means of a percent-reduction method on the 24-hr ADCav/T₂ time point, with homologous contralateral pixels used as reference. An SSD statistic was calculated for a series of percent-reduction values, and the minimum SSD defined the optimum percent-reduction threshold, which was determined to be 36% for ADCav and 68% for T₂.

We summed the lesion pixels from each slice to calculate the percent hemispheric lesion volume (% HLV) by dividing the number of abnormal pixels on each slice by the total number and multiplying by the slice thickness.

The same pixels were also used to calculate the average ADC of the lesion at each time point measured, and to prepare an image-processing mask that defined the spatial extent of the lesion. Reference pixels were used to calculate a reference average ADC, and an average_ADC-ratio change for the lesion pixels. Masks of the ADC-determined lesion at 3 and 24 hr were applied to the acute and subacute T₂ and CBFi maps. The average value of CBFi and T₂ at each time point was then calculated for the area of the ADC-identified lesion and the homologous reference area. Thus, the same pixels were analyzed for the temporal evolution of the ADC, CBFi, and T₂ at all time points measured. The 3-hr time point was chosen because it was the last time point measured during the acute phase of lesion development. The 24- and 48-hr time points were chosen because it was the point of maximum vasogenic edema development and the reference time point for TTC (29) histological analysis.

Statistical Analysis

All results are presented as mean ± SEM. The Wilcoxon matched-pairs test was used to compare the variables. Test results were marked as significant at P < 0.05.

RESULTS

The physiological parameters (e.g., body temperature, arterial blood pressure, pH, PaCO₂, and PaO₂) before and after injection of the microspheres were not significantly different (paired t-test, data not shown).

Figure 1 illustrates the temporal evolution of ADCav. An area of reduced ADCav appeared within 1 hr postinjection (data not shown (30)) and then slowly grew throughout the acute period (up to 3 hr), with a concomitant drop in the ADCav. After 24 hr, the size of the ADCav-detected lesion was significantly larger than at 3 hr. At 48 hr, the ADCav of the lesion started to renormalize. Consequently, the ADCav change is not the most sensitive method of lesion detection at this time point. Figure 1 also illustrates the temporal evolution of the perfusion deficit. In this case, an area of reduced CBFi in the posterior part of the affected hemisphere appears immediately after the injection of the microspheres. The size of the reduced CBFi area remained relatively constant throughout the acute period. At 24 hr, there appeared to be an increase in the area of CBFi reduction, which remained at a comparable size at the 48-hr time point. The T₂ maps depicted no lesions in the acute data set (up to 3 hr). A lesion is visible at 24 hr, which coincides with the size and location of the ADCav-apparent lesion. The T₂-defined lesion remained at a comparable size at the 48-hr time point. Figure 1 shows that the size and location of the histologically-defined lesion agreed well with the size and location of the ADCav- and T₂-detected lesion. The histology results (slice 5 from a typical animal appears in Fig. 1) illustrate the heterogeneous, multifocal nature of the resulting lesion in this model.

The multifocality and heterogeneity of the lesions are also apparent in the MR images shown in Fig. 2. This figure, which shows the ADCav images for three different animals at different time points after microsphere injection, shows more than one lesion focus in each MR image.
These images also demonstrate the relative heterogeneity of the lesions in this model as compared to the MCAO model.

Figure 3 shows the temporal evolution of the ADC$_{av}$- (squares) and $T_2$-determined (circles) lesion volume for all of the animals. An area of reduced ADC$_{av}$ (3.4% ± 0.9% HLV) appears 1 hr post-microsphere injection ($N = 20$). The area of reduced ADC$_{av}$ grows in the acute phase (up to 3 hr ($N = 20$)). At 12 hr, the area of reduced ADC$_{av}$ is significantly ($P < 0.01$) larger than at 3 hr (16% ± 2% HLV ($N = 8$) vs. 5% ± 1% HLV ($N = 20$), respectively. At 24 hr, the area of reduced ADC$_{av}$ is still significantly ($P < 0.001$) larger (15 ± 2% HLV; $N = 17$) than at 3 hr. At 48 hr ($N = 5$), the ADC$_{av}$ of the lesion almost completely renormalizes. Figure 3 also shows the $T_2$-lesion development with time for all the animals. No $T_2$ lesion was apparent during the acute period ($N = 20$). At 12 hr, a $T_2$-lesion of 9% ± 2% HLV (significantly larger than at 3 hr, $P < 0.01$) was measured ($N = 8$), increasing significantly ($P < 0.01$ in comparison to 12 hr, $P < 0.001$ in comparison to 3 hr) to 14% ± 2% HLV at 24 hr ($N = 17$), and declining to a value of 9% ± 4% HLV ($N = 5$) at 48 hr. Only dead animals were excluded from the study. The average pixel values of ADC$_{av}$ in the areas defined as lesion declined to 0.46 ± 0.01 × 10$^{-5}$ cm$^2$/s by 1 hr postinjection, and remained at this level throughout the acute period (up to 3 hr). At 12 hr, the average pixel value of ADC$_{av}$ in the lesion area declined significantly ($P < 0.01$) to 0.41 ± 0.01 × 10$^{-5}$ cm$^2$/s and remained near this value (0.42 ± 0.01 × 10$^{-5}$ cm$^2$/s) at 24 hrs (not significantly different from the ADC$_{av}$ at 12 hr, but still significantly decreased from 3 hr ($P < 0.001$)). At 48 hr, the average pixel values of ADC$_{av}$ in the lesion started to renormalize, attaining a value of 0.44 ± 0.02 × 10$^{-5}$ cm$^2$/s.

The values of the CBF$_i$ in the areas identified as lesion at 24 hr (Fig. 4a, filled squares) declined immediately after the microsphere injection to 74% ± 3% of the contralateral values, and declined steadily to 68% ± 2% of the contralateral values ($P < 0.05$) by 3 hr postinjection ($N = 20$). The CBF$_i$ at 12 hr ($N = 8$) declined significantly ($P < 0.05$) to 55% ± 4% of the contralateral values and attained a value of 57% ± 2% at 24 hr ($N = 17$) (significantly different from the 3-hr value, $P < 0.001$) and 53% ± 9% (not significantly different from the 3-hr value) at 48 hr ($N = 5$). However, for the areas that were identified as lesion at 3 hr postinjection (Fig. 4a, open circles), the values of CBF$_i$ declined immediately to 68% ± 4%, and then reached a value of 54% ± 2% at 3 hr postinjection.

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**FIG. 1.** Time course of microsphere-induced lesion development for a representative animal, as depicted in calculated ADC$_{av}$, CBF$_i$, and $T_2$ maps. A representative histological slice obtained by TTC staining is also shown for comparison purposes. The animal received a dose of 1000 microspheres. The ADC$_{av}$-apparent lesion increases in size up to 24 hr post-microsphere injection and then declines due to renormalization of the ADC$_{av}$ value (which reduces the number of pixels that exceed the ADC$_{av}$ threshold used to estimate the ADC$_{av}$ lesion volume). $T_2$ maps illustrate lesion areas at 24 and 48 hr postinjection. Note the good agreement in terms of lesion size and placement among the ADC$_{av}$ maps, $T_2$ maps, and histology.

**FIG. 2.** ADC$_{av}$ maps of three different animals at different time points after microsphere injection, illustrating the multifocality and relative heterogeneity of the lesion in this model: (a) acquired at the 12-hr time point; (b and c) acquired at 24 hr.
values. At 48 hr (P < 0.001), 57% ± 3% at 12 hr (not significantly changed from the 3-hr value), 59% ± 3% at 24 hr (not significantly changed from the 3-hr value, but significantly changed from the injection time value; P < 0.005), and 45% ± 4% at 48 hr postinjection. It should be noted that the area determined as lesion at 3 hr is significantly smaller than that determined as lesion at 24 hr, and probably consists of the most severely affected pixels of the lesion area. There was a small reduction in CBF, before microsphere injection, as discussed previously (18,31,33), and therefore we selected the contralateral values as reference.

The values of $T_2$ in the areas identified as lesion at 24 hr (Fig. 4b, filled squares) remained in the range identified before injection until they reached a value of 123% ± 3% of the contralateral values at 3 hr postinjection (significantly different from the baseline value, $P < 0.005$) ($N$ = 20). At 12 hr, the $T_2$ values increased significantly ($P < 0.001$) to 171% ± 4% of the contralateral values ($N$ = 8). The values at 24 hr ($N$ = 17) were significantly different from the 3-hr values ($P < 0.001$) and the baseline value ($P < 0.001$), and reached 182% ± 5% of the contralateral values. At 48 hr ($N$ = 5), there was a decline in the $T_2$ values in the lesion volume, and a value of 140% ± 20% of the contralateral values (not significantly different from baseline) was achieved. The values of $T_2$ in the areas identified as lesion at 3 hr (Fig. 4b, open circles) increased within the first 3 hr postinjection to a value of 115% ± 3% of the contralateral values ($P < 0.001$). By 12 hr, however, the $T_2$ in the volume identified as lesion by ADC$_{av}$ at 3 hr increased significantly, reaching a value of 170% ± 10% ($P < 0.001$). At the 24-hr time point, the average $T_2$ increase in the lesion was 189% ± 4% (significantly different from 3 hr, $P < 0.001$) and by 48 hr, $T_2$ decreased to 150% ± 30%.

**DISCUSSION**

Both the acute-stage ADC$_{av}$ maps (Fig. 2) and the histology show that the lesions were multifocal and relatively heterogeneous. There was a good agreement with the areas determined as lesion in the TTC slides and the 12- and 24-hr ADC$_{av}$ and $T_2$ maps. However, as expected, the ADC$_{av}$ maps showed much better sensitivity than the $T_2$ maps for delineating the ischemic process during the acute phase (6,7,21). At 24 hr there was good agreement between the ADC$_{av}$ maps and histology. Disagreement between the lesion evaluations by the MRI techniques and TTC may

![Graph showing lesion volume development over time](image)

**FIG. 3.** ADC$_{av}$ (squares) and $T_2$ (circles) lesion size development (expressed as percentage of the hemisphere) with time. The ADC$_{av}$ lesion appears 1.0 hr postinjection, and by the 3-hr time point the volume of ADC reduction increases to 5% ± 1% (mean ± SEM) of the brain hemisphere. The lesion volume increased significantly ($P < 0.01$) to 16% ± 2% of the hemisphere volume at the 12-hr time point, while at 24 hr the lesion (15% ± 2% of the hemisphere) was also significantly larger ($P < 0.001$) than at 3 hr. Note that the apparent reduction of ADC-defined lesion at 48 hr is due to renormalization of the ADC above the threshold used to define the lesion. The $T_2$ lesion appears only at the 12-hr time point, and increases significantly in size to 14% ± 2% HLV at 24 hr ($P < 0.01$ as compared to 12 hr, $P < 0.001$ as compared to 3 hr). Note that the apparent reduction of $T_2$-defined lesion at 48 hr is due to a decrease in $T_2$ (see Fig. 4b) relative to the fixed threshold used to define the lesion.

![Graph showing CBF ratio over time](image)

**FIG. 4.** a: Time course of CBF, of the pixels determined as lesion by a mask of the ADC$_{av}$ lesion at 3 hr (open circles) and 24 hr (filled squares) postinjection. The value of CBF, in the pixels determined as lesion at 3 hr declines more severely than in the pixels determined as lesion at 24 hr during the acute phase, but reaches similar value at the 12-hr time points and onward. Note that the area determined as lesion at 3 hr is significantly smaller than that determined as lesion at 24 hr, and probably consists of the most severely affected pixels of the lesion area. b: Time course of $T_2$ of the pixels determined as lesion by a mask of the ADC$_{av}$ lesion at 3 hr (open circles) and 24 hr (filled squares) postinjection. The value of $T_2$ in pixels determined as lesion at 3 or 24 hr postinjection did not increase significantly until 12 hr postinjection. From 12 hr onward, $T_2$ values in the 3- and 4-hr lesion areas increased significantly, and remained significantly elevated at 24 hr as well. A reduction in the $T_2$ values was observed at the 48-hr time point.
arise from partial volume averaging. Smaller, multifocal lesions, such as those that occur in this model, are more susceptible to the problem of volume averaging. Also, it should be considered that the TTC-stained image represents only the surface of the slice, whereas the ADCav and $T_2$ maps represent the average of the values throughout the whole thickness of the slice. The average size of the resulting lesion in this study compares well with that described by Miyake et al. (18), who detected (by TTC) an average lesion size of approximately 21% of the control hemisphere (as compared to 15% ± 2% HLV in the present work).

In the microsphere model, the ADCav-apparent lesion increased in size up to 24 hr postinjection, in contrast with the rat MCAO model, in which the lesion reached its maximum size 3–4 hr postocclusion. However, the pattern of lesion development followed roughly the same outlines as in the MCAO model—only the time scale of the changes was different. In the MCAO model, Welch et al. (32) found that the maximal ADCav decrease occurs at approximately 6 hr, with a $T_2$ increase maximizing between 24 and 48 hr post-MCAO (32). In contrast, in the microsphere model, the ADCav decrease maximizes between 12 and 24 hr, while the $T_2$ increase maximizes at around 24 hr. Fukuchi et al. (20) also found, based on $T_2$-defined lesions, that the temporal development of the microsphere model is different from the time course determined in a major artery occlusion model or a temporary ischemia model (20).

Another unique characteristic of the microsphere model is the multifocal and heterogeneous nature of the developing lesions. This multifocality was also observed by Miyake et al. (18), who found necrotic areas of various sizes and shapes distributed in various brain regions. This multifocality results in more areas that have reduced perfusion with varying degrees of collateral flow. These areas are initially not infarcted, which helps us determine which compromised areas will proceed to infarction and which are potential candidates for therapy. Additionally, the multifocal nature of the lesions and the slow development of lesions to their final size give us a relatively extended temporal evolution of the microsphere model, which mimics the clinical situation better than the permanent MCAO model (35,36). Also, since the lesion develops more slowly, there is a larger area of tissue that is affected to varying degrees but is not infarcted, and may serve as a good location for assessment of therapy. The slower development of ischemic lesions allows investigators more time to test possible therapies. This implies that in terms of time-course evaluations of lesions, the microsphere-induced embolic stroke model mimics the clinical situation better than the permanent MCAO model (35,36). Also, since the lesion develops more slowly, there is a larger area of tissue that is affected to varying degrees but is not infarcted, and may serve as a good location for assessment of therapy. The slower development of the lesion mimics the clinical case (37), where a deficit that lasts <24 hr is considered to be a transient ischemic attack (TIA). One may therefore speculate that this model may be as useful for drug testing and evaluation (excluding reperfusion drugs) as other well-documented and commonly used models, such as the rat MCAO model.

The microsphere model does not exactly simulate the clinical situation, since the microspheres cause a permanent occlusion that cannot be recanalized, spontaneously or interventionally. However, this model is still suitable
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