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**Spatiotemporal Characterization of Brain Infarction by Sequential Multimodal  
MR Imaging Following Transient Focal Ischemia in a Rat Model of Intra-  
Arterial Middle Cerebral Artery Occlusion**

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*Key points*

- Nitinol microwire is compatible with MR imaging allowing spatiotemporal echaracterization of brain infarction in rat.
- Selective placement of microwire in middle cerebral artery allows a complete artery occlusion in 75% with a strict control of the ischemia and reperfusion phase.
- A diffusion/perfusion mismatch during arterial occlusion is observed in 77% of rats.

## **Abstract**

*Objectives* To assess spatiotemporal brain infarction evolution by sequential multimodal magnetic resonance (MR) imaging in model of acute stroke in rats by selective intra-arterial middle cerebral artery (MCA) occlusion

*Materials and methods* A 0.007-inch microwire was selectively placed in the MCA from the ventral tail artery in 16 consecutive rats during 90 minutes occlusion. Longitudinal 7-T MR imaging, including angiography, diffusion, and perfusion was performed during ischemia, immediately after reperfusion, 3 hours and 24 hours after subsequent reperfusion.

*Results* MCA occlusion was complete in 75% and partial in 18.7%. Hypoperfusion (mean  $\pm$  SD) was observed in all animals during ischemia ( $-59 \pm 18\%$  of contralateral hemisphere, area  $31 \pm 5$  mm<sup>2</sup>). Infarction volume (mean  $\pm$  SD) was  $90 \pm 64$  mm<sup>3</sup> during ischemia and  $57 \pm 67$  mm<sup>3</sup> at 24 hours. Brain infarction was fronto-parietal cortical in 5 animals (31%), striatal in 4 animals (25%), and cortico-striatal in 7 animals (44%) at 24 hours. All rats survived at 24 hours.

*Conclusion* This model of acute stroke in rats by selective intra-arterial MCA occlusion is suitable to neuroprotection studies because of possible acute and close characterization of spatiotemporal evolution of brain infarction by MR imaging techniques, and evidence of ischemic penumbra, the target of neuroprotection agents. However, optimization of the brain infarct reproducibility needs further technical and neurointerventional tools improvements.

**Keywords** Stroke model, Endovascular, Middle cerebral artery occlusion, Diffusion/Perfusion mismatch, Neuroprotection



## **Abbreviations and acronyms**

MR Magnetic resonance

MCAO Middle cerebral artery occlusion

ECA External carotid artery

ICA Internal carotid artery

MCA Middle cerebral artery

TOF Time of flight

DWI diffusion-weighted imaging

PWI Perfusion-weighted imaging

MRA Magnetic resonance angiography

## **Introduction**

Translational research in stroke treatment is lagging due to the absence of an outstanding animal model. Existing models of focal ischemia are riddled with technical limitations that impair the translation of scientific insights to human physiology because 1) they misrepresent the subset of strokes eligible for clinical intervention in humans 2) the delicate technique and traumatic surgery often results in partial occlusion or complications. To date, the intraluminal thread occlusion of the MCA is the most widely used model of focal cerebral ischemia in rats (suture model) [1]. A nylon monofilament is negotiated through the external carotid artery (ECA) and compacted at the termination of the internal carotid artery (ICA). The pellet obstructs the anterior middle cerebral artery (ACA), the middle cerebral artery (MCA) and the posterior communicating artery promoting massive infarcts [1-3]. Technical issues arise in a majority of the cases such as partial occlusion and/or spontaneous reperfusion, which occurs in 25% of cases [4,5], or subarachnoid hemorrhage due to arterial perforation, seen in 30% of cases [4]. In addition, extensive cervical surgery requires permanent ligation of multiple branches of the ECA and induces a bias in neurological outcomes and behavioral tests [6,7].

Emerging endovascular technologies provide new possibilities for cerebral intervention in acute stroke, and thrombectomy is now the reference of care in stroke with large vessel occlusion in association with thrombolysis within 6 hours [8]. However, around 50% of treated present a non favorable outcome at 3 months despite successful reperfusion; in fact, reperfusion injury and no-reflow phenomenon could limit recanalization benefit. Intensive monitoring of reperfusion in order to understand the underlying mechanisms and evaluation of new neuroprotective agents is necessary. A new model of transient focal ischemia in a rat was recently described

using minimally invasive technique to selectively occlude the MCA with a microwire navigated from the ventral tail artery (microwire model) [9].

The aim of our longitudinal 7-T MR imaging study was to characterize the acute development of the ischemic infarct and ensuing reperfusion, and follow the evolution of the ischemic lesions during 24 hours. The exhaustive MR imaging protocol includes the systematic acquisition of T2 spin-echo, angiography, diffusion-, perfusion-weighted imaging, and T2 gradient-echo (T2\*) during the MCA occlusion, immediately after reperfusion, 3 hours and 24 hours after reperfusion.

## **Materials and methods**

### **Animal model**

All procedures were approved (N°01099.02) by our Institutional Review Board (Comité Régional d'Éthique pour l'Expérimentation Animale Rhône-Alpes of the CNRS), in compliance with French (decree 2013-118) and European Community (2010/63/UE) guidelines.

Sixteen consecutive young adult male Sprague-Dawley rats (Janvier, Le Genest Saint Isle, France; mean, 403 g; range, 320-470) were anaesthetized with 3% isoflurane in ambient air. To minimize suffering, 0.05 mg/kg subcutaneous buprenorphine was administered immediately after the beginning of anesthesia. Body temperature was continuously maintained at  $37\pm 1^{\circ}\text{C}$  by a feedback-regulated heating pad. The procedure was performed as originally described [9]. Briefly, the ventral tail artery was exposed by cutting the fascia and puncturing the artery. A 0.007-inch Hybrid microwire (Balt Extrusion, Montmorency, France) was advanced through the thoracic descending aorta.

Subsequent steps were performed using flat-panel x-ray system (Allura Xper XD20, Philips Medical Systems; 80 mAs, 120 kVp). A 0.020-inch Marathon microcatheter (Covidien, Irvine, California) carrying a microwire was introduced and advanced to the aorta and common carotid artery. Next, the microwire was navigated to a tip position distal to the bifurcation of the MCA to occlude the MCA. Ischemia was maintained for 90 minutes in all animals. Reperfusion was achieved by withdrawing the microwire under MR imaging control.

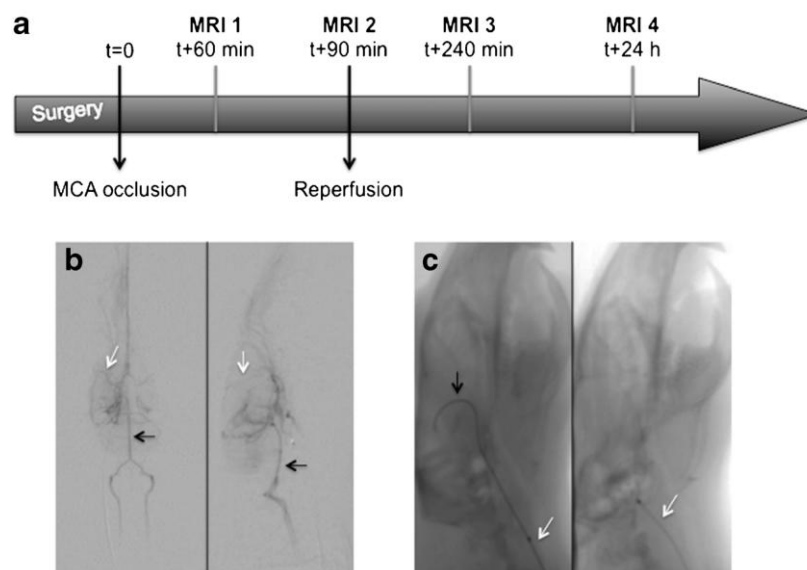
Thereafter, the microwire was retracted together with the microcatheter out of the animal and the tail artery was tightened and the incision was closed. The animals



were returned to their cages with food and water *ad libitum*. At 3 and 24 hours after MCA recanalization, the animals were reanesthetized for additional MR imaging.

### Protocol

Four parameters were included in the 30 minutes MR imaging protocol: T2 spin-echo, time of flight (TOF) angiography, diffusion-weighted imaging (DWI), perfusion-weighted imaging (ASL), and T2 gradient-echo (T2\*). Four sequential sets of MR imaging were systematic acquired: during ischemia, immediately after reperfusion, 3 hours and 24 hours following reperfusion. No DWI was acquired at 24 hours. The study MR imaging protocol is illustrated in Figure 1.



**Figure 1.** **A**, Time line for the experimental protocol with serial MR imaging session during ischemia, immediatly after reperfusion, and 3 and 24 hours after subsequent reperfusion. **B**, Digital subtraction angiography, frontal and lateral projections, with contrast injected selectively in the internal carotid artery with the Marathon microcatheter showing the course of the middle cerebral artery (white arrows). The basilar artery (black arrows) is opacified by retrograde manner. **C**, Microguidewire (black arrow) was placed selectively in the distal portion of the middle cerebral artery for 90 minutes. Microcatheter (white arrow) was

positioned in the common carotid artery..Thereafter, the microwire was retracted and reperfusion occurred.

### **MR imaging**

All animals underwent MR imaging using a Bruker Biospec 7T/12 centimeters horizontal magnet equipped with Paravision 5.0. A birdcage head-coil of 72 millimeters (mm) inner diameter was used for radiofrequency transmission and a 25 mm diameter surface coil was used for reception. The rats were placed in a cradle equipped with a stereotaxic holder, an integrated heating system to maintain body temperature at  $37\pm 1^{\circ}\text{C}$ , and a pressure probe to monitor respiration. Imaging was performed in the axial plane and included the following sequences:

T2-weighted spin-echo images were acquired using a RARE sequence with matrix =  $256 \times 128$ , FOV =  $3 \times 1.5 \text{ cm}^2$ , 15 slices with slice thickness of 1 mm without gap, covering the entire MCA territory, TE/TR = 60.3/3500 milliseconds (ms), 4 averages, RARE 8 and fat-suppression. The infarct volume at 24 hours after reperfusion was evaluated with T2 sequence.

For TOF angiography, a coronal 2D-inflow gradient echo sequence was performed with the following parameters: field of view (FOV) =  $37 \times 25 \text{ mm}$ , matrix size =  $256 \times 128$  pixels, in-plane spatial resolution =  $145 \times 195 \mu\text{m}^2$ , TE = 5 ms, TR = 25 ms, flip angle =  $90^{\circ}$ , number of averages = 2, bandwidth = 40 kHz; a moving saturation pulse was also used. A number of 80 slices of 0.3 mm thickness and 0.15 inter-slice distances were acquired during 6 minutes and 24 seconds scan time.

DWI was performed using echo planar imaging (EPI) with the same FOV and slice characteristics as T2-weighted images, with matrix =  $128 \times 128$ , TE/TR = 25/2000 ms and 3 b-values (0, 1500, and  $3000 \text{ s/mm}^2$ ).

ASL was performed with a single-slice pulsed arterial spin labeling method. ASL was performed using a flow-sensitive alternating inversion-recovery echo planar imaging (FAIR-EPI) sequence with matrix 96 x 48, FOV = 2.61 cm<sup>2</sup>, inversion recovery time (TIR) = 40 to 2.500, number of TIR values = 22, recovery time = 10.000 ms, TE/TR = 10/18.000 ms.

T2-weighted gradient-echo (T2\*) imaging were acquired using a 2D multi-echo gradient sequence with 6 TE (3.3, 7.52, 11.78, 16.03, 20.28, and 24.53 ms), TR = 713.4 ms, flip angle 25°, 4 averages, FOV = 3 x 1.5 cm<sup>2</sup>, with matrix = 192 x 96, giving a pixel dimension of 0.156 x 0.156 μm<sup>2</sup>, 15 slices of 1 mm thickness were acquired.

T2 spin-echo, angiography, diffusion, and T2 gradient-echo (T2\*) covered the entire MCA territory, while perfusion was calculated using the central slice only.

### **Image analysis**

All MRI scans were analyzed and scored independently by two investigators (B.G., J.B.L.) using the MIPAV software [10]. MCA occlusion was assessed by both source images and maximum intensity projection of TOF angiography. The scoring system is as described: complete occlusion (undetectable flow along the whole MCA route), partial occlusion (decreased flow signal), and no occlusion (normal flow signal). ADC maps (mm<sup>2</sup>/s) were calculated by fitting MR signal curves to a monoexponential model function on a pixel-by-pixel basis, using in-house software written in Matlab (MathWorks, Natick, MA, USA). ASL images were analyzed with the ASL\_Perfusion\_Processing macro from the Paravision 5.1 platform (Bruker, Ettlingen, Germany), using a 7-T blood T1 value of 2.200 ms [11]: cerebral blood flow (CBF, in ml/[minx100 g]) was derived from the non-selective and selective T1

maps according to  $CBF = f \cdot T1_{non-selective} / T1_{blood} \cdot (1/T1_{selective} - 1/T1_{non-selective})$ , where  $f$  is the blood-brain partition coefficient i.e. the ratio between water concentration per gram of brain tissue and per mL of blood, estimated at 90 mL/100 g [12]. Diffusion and perfusion lesions were manually outlined on ADC and CBF maps, respectively. T2\* maps were reconstructed after the acquisition using the Image Sequence Analysis tool from Bruker's ParaVision software. The T2\* relaxation time was expressed in ms.

Diffusion, perfusion lesions and final infarct volume at 24 hours were manually outlined on ADC, ASL, and T2 maps, respectively, using MIPAV software.

### **Behavioral examination**

Sensorimotor function evaluation was measured 24 hours after reperfusion (F.C. blinded to MR imaging data), using the previously reported sensorimotor performance score on a scale of 0 to 9 (Bederson scale) and 0 20 points (van der zijden scale) [13,14]. The Bederson scale is a global neurological assessment including forelimb flexion, resistance to lateral push and circling behavior. This scoring scale is a simple way to reveal basic neurological deficits. The van der Zijden scale was based on: (1) spontaneous activity during 1 minutes, (2) gait disturbances such as circling toward the paretic side, (3) degree or absence of pathological postural reflexes when held by tail, (4) degree of asymmetry in resistance between left and right lateral push, (5) degree of asymmetry in grasping strength onto a rod between left and right forepaw, and (6) degree or absence of forelimb placing onto a table rim after stimulation of the vibrissae. Higher scores reflect greater deficits.

### **Statistical analysis**

Nominal parameters (ADC, ASL and T2 volumes) are reported as mean±standard deviation (SD), while neuroscores are reported as median and range. Hypoperfusion is reported as a percentage value of contralateral hemisphere. Perfusion-diffusion mismatch area was expressed as mean ± SD. The correlation was calculated with a SPSS v21 by using a Spearman rank-order correlation.

## Results

### Work flow timing

The workflow timing results are summarized in Table 1. Mean time between anesthesia induction and MCA occlusion was 54±12 minutes (mean time induction-tail puncture 7 minutes and mean time between tail puncture-MCA occlusion was 48 minutes). Mean time of MCA occlusion was 87±19 minutes (range, 35-108).

<b>Rat</b>	<b>Induction-occlusion</b>	<b>Delay Ischemia</b>	<b>Occlusion-DWI 1</b>	<b>Occlusion-DWI 2</b>	<b>Occlusion-DWI 3</b>
<b>1</b>	57	90	45	110	407
<b>2</b>	47	90	42	109	324
<b>3</b>	55	98	77	107	310
<b>4</b>	70	90	41	101	348
<b>5</b>	72	95	34	111	285
<b>6</b>	67	108	78	120	326
<b>7</b>	49	104	86	122	318
<b>8</b>	67	50	25	47	339
<b>9</b>	45	100	83	119	288
<b>10</b>	43	93	72	120	286
<b>11</b>	67	90	42	107	303
<b>12</b>	56	93	43	123	296
<b>13</b>	49	35	22	91	NA
<b>14</b>	36	82	50	94	NA
<b>15</b>	37	90	71	103	289
<b>16</b>	47	82	47	93	NA
<b>Mean±SD</b>	<b>54±12</b>	<b>87±19</b>	<b>54±21</b>	<b>105±19</b>	<b>317±34</b>

Data shown are minutes. The third session of MR imaging was not performed for rat

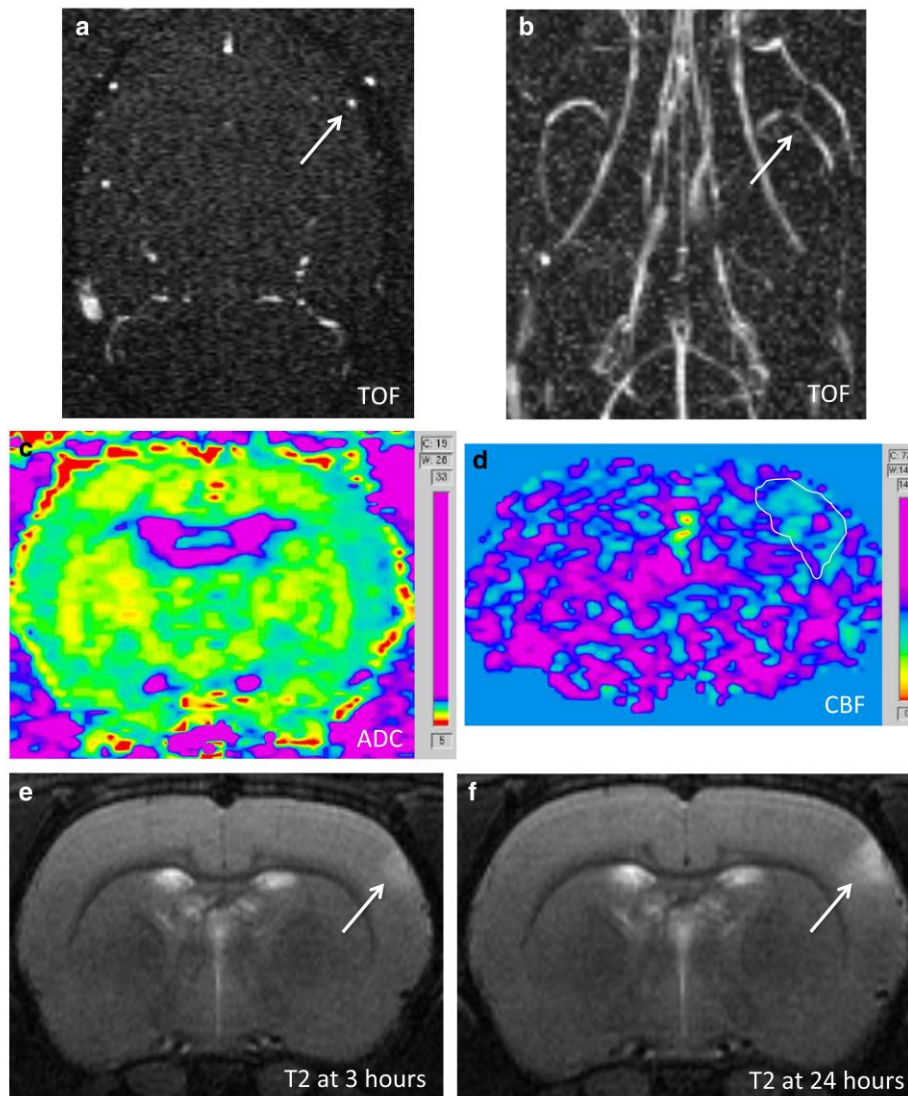
13, 14, and 16.

SD, standard deviation.

**Table 1.** Workflow timing.

### MCA occlusion status

The microguide was successfully placed in the distal MCA (3 right side and 13 left side) in all animals. After microwire placements, TOF angiography revealed complete MCA occlusion in 12 animals (75%), partial MCA occlusion in 3 animals (18.7%), and failure in 1 animal (6.3%, Figure 2). In all cases, we observed normal signal in distal MCA branches. The ipsilateral anterior cerebral artery was patent in all cases.



**Figure 2.** Multislice MR imaging of endovascular stroke in rats after failure MCA occlusion. **A** and **B**, MR angiography with TOF acquisition showing the patency of the left MCA (arrow). **C**, Diffusion (ADC map) showing no significant lesion during ischemia. **D**,

Perfusion (ASL map) showing cortical hypoperfusion. **E** and **F**, T2 map at 3 and 24 hours after reperfusion demonstrating small focal cortical infarction.

Immediately after reperfusion and at 24 hours, MRA revealed a patent MCA in all cases. At reperfusion, 8 partial recanalizations and 7 complete recanalizations were observed, and 1 partial recanalization and 15 complete recanalization at 24 hours.

### **Diffusion and perfusion results during microwire placement and after withdrawal**

The main results of MR imaging acquisition are summarized in Table 2.

	<b>ADC (mm<sup>3</sup>)</b>	<b>ASL (%)<sup>a</sup></b>	<b>ASL (mm<sup>2</sup>)<sup>b</sup></b>	<b>T2</b>
<b>During 90-minutes of ischemia</b>	90 ± 64	-59 ± 18	30 ± 5	-
<b>At reperfusion</b>	60 ± 55	-63 ± 17	22 ± 14	-
<b>3-hours after reperfusion</b>	38 ± 19	-48 ± 17	23 ± 11	-
<b>24-hours after reperfusion</b>	-	+10 ± 77	12 ± 6	57 ± 67

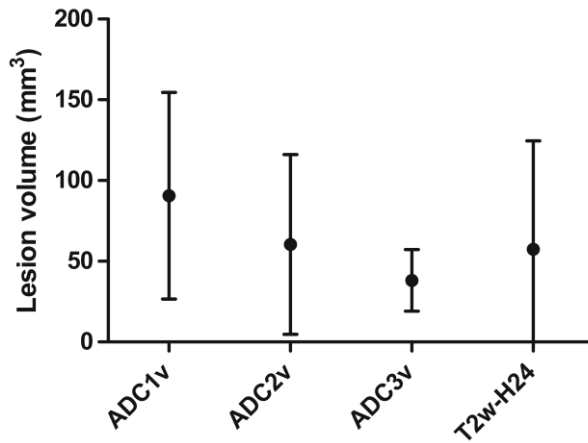
Data are expressed in mean ± standard deviation.

<sup>a</sup>ASL are expressed relatively to the mirror perfusion in the contralateral hemisphere.

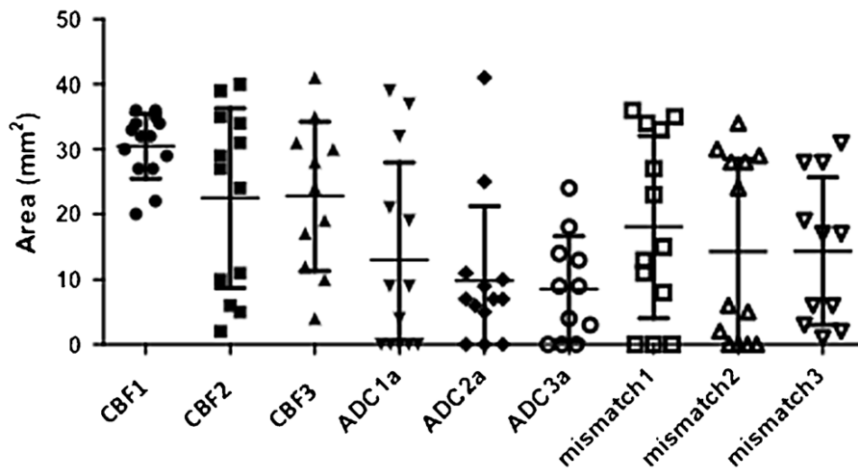
<sup>b</sup>Area of the hypoperfusion on ASL map.

**Table 2.** Main results of MR imaging acquisition.

Figure 3 shows the infarct volume during ischemia (ADC1v), immediately after reperfusion (ADC2v), 3 hours after reperfusion (ADC3v). A progressive decrease of ADC volume was observed (ADC1v = 90 ± 64, ADC2v = 60 ± 55, ADC3v = 38 ± 19, and T2 at 24 hours = 57 ± 67 mm<sup>3</sup>). Well-demarcated focal ADC lesions were observed in the cortex, striatum, or cortico-striatal in 12 animals, while the volume of ADC lesions were very small (< 10 mm<sup>3</sup>) in 4 animals. Figure 4 shows the evolution of ADC lesion area, hypoperfusion, and mismatch at the level of PWI acquisition during ischemia (map 1), reperfusion (map 2), and 3 hours after (map 3).



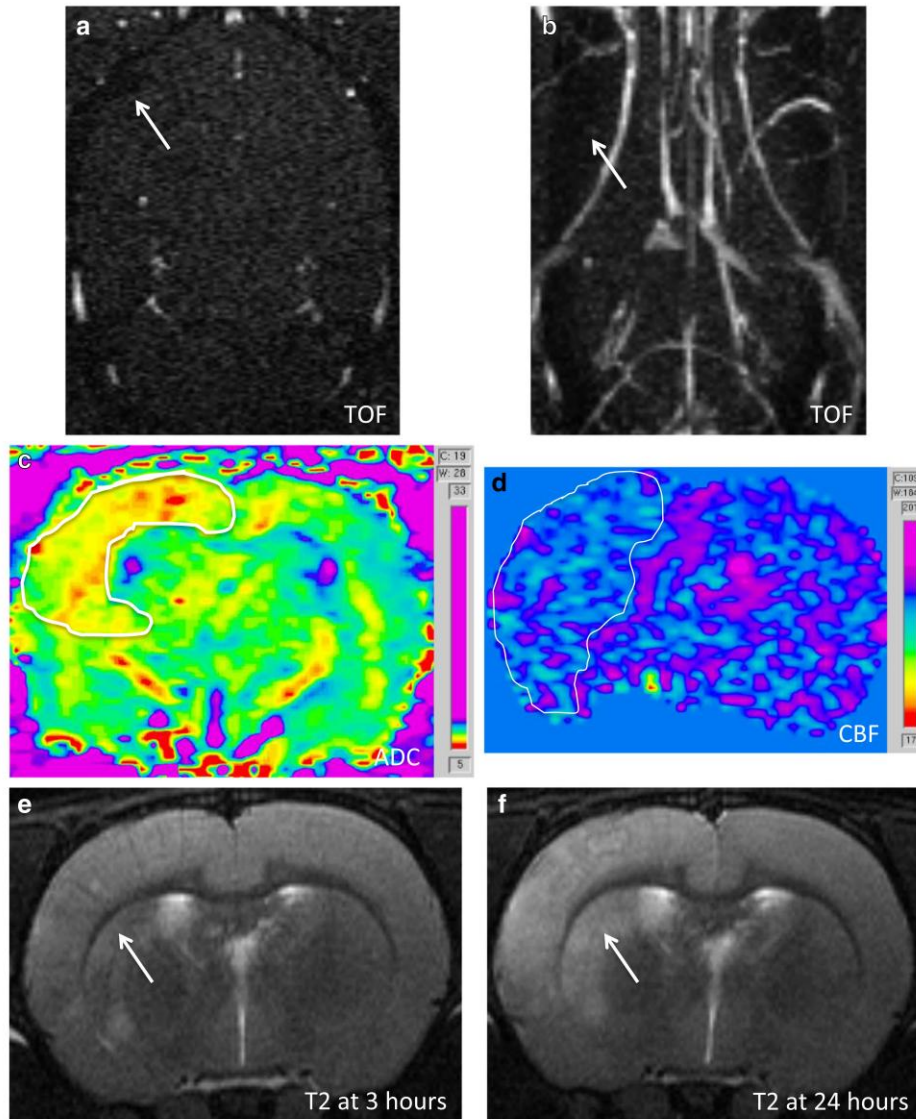
**Figure 3.** Evolution of infarct volume (expressed as mean  $\pm$  SD) during ischemia (ADC1v), at reperfusion (ADC2v), 3 hours after reperfusion (ADC3v), and 24 hours (T2 w-H24).



**Figure 4.** Evolution of lesion area (expressed as mean  $\pm$  SD) at the level of perfusion acquisition (1 slice) during ischemia (map 1), reperfusion (map 2), and 3 hours after (map 3). ASL was performed with a single-slice pulsed arterial spin labeling method (area), whereas ADC and T2 were performed with a multi-slice acquisition (volume). One, 3, and 5 animals were excluded in ASL1, ASL2, and ASL3 respectively.

After MCA guide placement, ASL confirmed hypoperfusion in all animals (PW11,  $-59 \pm 18\%$  of contralateral hemisphere, area  $31 \pm 5 \text{ mm}^2$ ; 14 rats). Interestingly, pixel-by-pixel analysis revealed a perfusion mismatch during arterial occlusion in 77% of cases (10 out of 13 rats). Figure 5 illustrates the DWI-ASL patterns observed during ischemia, and infarct evolution at 3 hours and 24 hours after reperfusion.





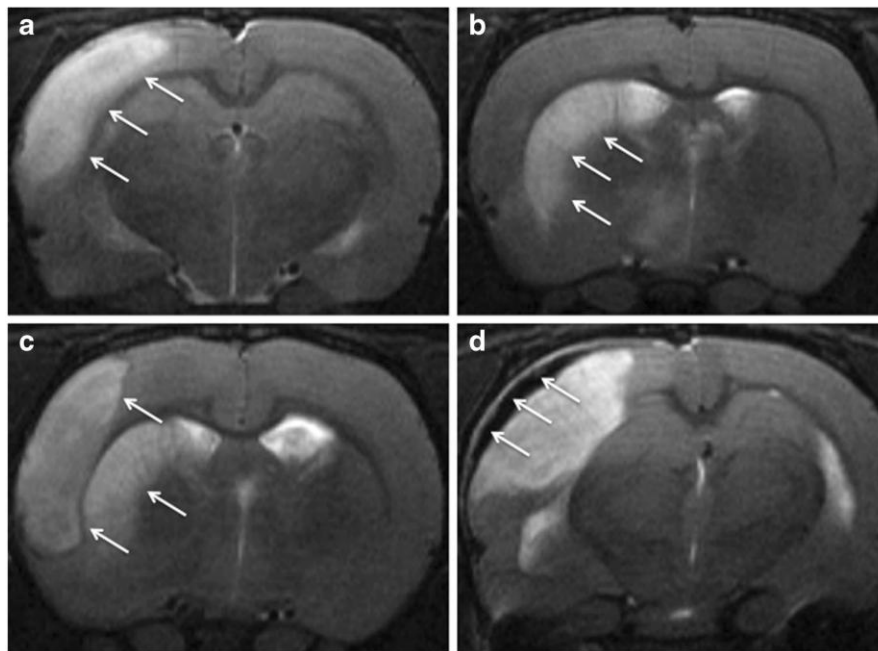
**Figure 5.** Multislice MR imaging of endovascular stroke in rats. **A**, MR imaging analysis showed T2 spin-echo signal loss throughout the right distal MCA lumen. **B**, MRA (MIP reconstruction) with TOF acquisition showing the complete selective occlusion of the right MCA. **C**, Perfusion (ASL1) map showing large cortico-striatal perfusion defect. **D**, Diffusion (ADC1 map) showing deep infarction.

At the same level as PWI1 acquisition, 5 rats demonstrated immediate brain reperfusion following guidewire withdrawal, whereas others animals presented persistent hypoperfusion despite arterial recanalization. This evolution was not

correlated with the delay of ischemia, neuroscore, volume of ADC1v, ADC2v, ADC3v, T2 24 hours, area of ASL or ADCv.

### **Reproducibility of the infarct region**

The arterial occlusion was systematically performed at the level of distal MCA. Figure 6 illustrates the pattern of infarct zones at 24 hours. It was localized at the level of fronto-parietal cortex in 5 animals (31%), striatum in 4 animals (25%), and in cortico-striatal area in 7 animals (44%) at 24 hours.



**Figure 6.** Pattern of stroke lesion in a rat model of intra-arterial MCA occlusion. **A**, Fronto-parietal cortical infarction. **B**, Infarction of the striatum. **C**, Cortico-striatal infarction. **D**, Acute extra-cerebral hematoma.

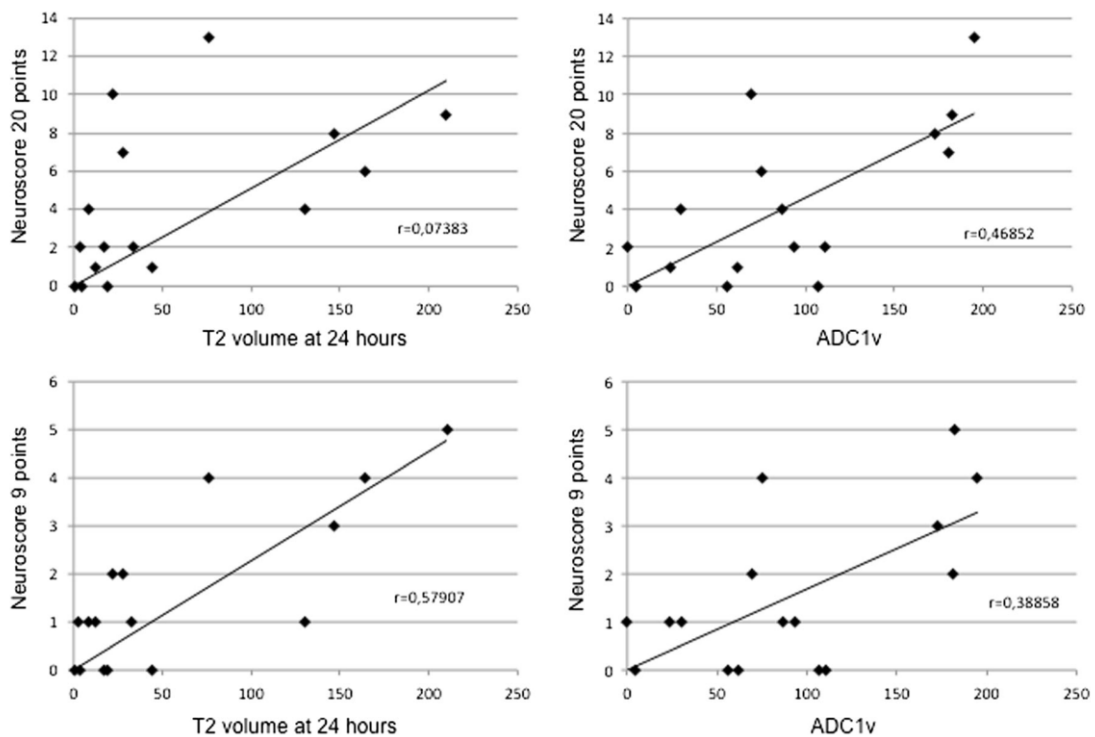
### **Hemorrhagic complication and mortality**

At 24 hours, T2\* highlighted an extra-cerebral hematoma around the microguide placement in 2 animals (Figure 6D), and an intracranial hematoma in 1 animal. A total

of 3 out of 16 rats (18.7%) presented a hemorrhage complication at 24 hours. No mortality was observed throughout the study.

### Behavioral evaluation

The median behavioral score 24 hours after the onset of ischemia was 1 (range, 0-5) and 3 (range, 0-13), respectively, on the 9-point and 20-point scales. Figure 7 shows the correlation between neuroscore and T2 volume at 24 hours, and a good correlation was observed between ADC1v, T2 volume and behavioral results (ADC1v-neuroscore 9 points:  $r=0.49$ ,  $p=0.07$ ; ADC1v-neuroscore 20 points:  $r=0.56$ ,  $p=0.04$ . T2 volume-neuroscore 9 points:  $r=0.70$ ,  $p=0.01$ ; T2 volume-neuroscore 20 points:  $r=0.58$ ,  $p=0.03$ ).



**Figure 7.** Correlation between ADC1v, T2 volume and behavioral results. ADC1v-neuroscore 9 points:  $r=0.49$ ,  $p=0.07$ ; ADC1v-neuroscore 20 points:  $r=0.56$ ,  $p=0.04$ . T2 volume-neuroscore 9 points:  $r=0.70$ ,  $p=0.01$ ; T2 volume-neuroscore 20 points:  $r=0.58$ ,  $p=0.03$ .



## **Discussion**

Our study provides an in-depth multimodal MR imaging characterization of the spatiotemporal evolution of the brain infarction in a novel stroke model by selective intra-arterial MCA occlusion in the rat. We showed that a 0.007-inch microwire placement under fluoroscopic control allows a complete MCA occlusion in the majority of the cases (75%) with a subsequent strict control of the ischemia and reperfusion. This study is the first independent validation of the feasibility of the model after its original description by Arnberg et al [9].

### **Safety of microwire model**

The intra-arterial approach from the tail artery is truly minimally invasive and safe. In fact, only a small cutaneous incision (less than 1 cm) and artery puncture were required. In addition, the ligation of the tail artery at the end of the procedure was well tolerated because its distal part was vascularized by the 2 lateral tail arteries, as previously reported [9]. In our study, no ischemia of the tail was observed and the mortality rate was null at 24 hours. All rats quickly recovered and were able to move and feed without assistance when returned to their cages. Behavioral score is well correlated with infarct size at 24 hours. This intra-arterial technique is minutely traumatic thus provides an opportunity for longer follow-up, which may yield sensitivity for neurobehavioural analysis and neuroprotection studies. On the contrary, the mortality rate in the MCAO model was high. In an MR imaging study of MCAO, 4 of 19 (21%) rats died during the first 48 hours, which limited the investigative power of the study [15]. MCAO model requires an extensive cervical surgery and definitive ligation of several branches of the ECA, which is responsible for the loss of

up to 10% of the presurgical weight 3 to 4 days after [6]. This invariably affects the results of the behavioral study [7].

In this new model, the distal part of the MCA is selectively occluded by fluoroscopic guidance, whereas, in the MCAO model, the monofilament was advanced without visual control until its tip blocked the ICA termination, and so occluded the origin of the MCA and the ACA. An additional permanent occlusion of the ipsilateral internal or common carotid artery is necessary because of direct carotid approach. No occlusion of the ICA was performed in the microwire model during or after the MCA occlusion. In addition, in the suture model, vessel perforation may occur before any resistance is perceptible, and subarachnoid hemorrhage may reach up to 30%, [4] whereas we observed 18.7% with the microwire model. The lowest rate of hemorrhagic complication observed in this study is probably explained by a better control of the intra-arterial device.

### **Efficacy of microwire model**

In our study, MCA was completely occluded in 75% of cases and partially in 18.7% with ipsilateral anterior cerebral artery patent in all cases. Importantly, the nitinol distal tip of microwire did produce discrete artifacts that may hamper analysis of the signal at the MCA. Transcranial doppler could be an interesting option to confirm the occlusion of the MCA. In the suture model, there may be a clear resistance of the filament although the MCA is still not completely occluded. Premature reperfusion was noted in 25% of the experiments whatever the type of filament (3-0 filament or silicone-coated 4-0 filament) [4]. However, continuous bilateral [4] or multi-site [16] laser-doppler flowmetry could confirm arterial occlusion.

We observed cerebral hypoperfusion in all cases with < 60% of contralateral hemisphere in 12 cases. Interestingly, immediate reperfusion was not obtained in all cases after withdrawal the guidewire. Only 36% of rats demonstrated brain reperfusion following MCA recanalization. These findings called the «no-reflow phenomenon» are well known in experimental and clinical practice [17,18]. The MR imaging compatibility of this model, based on MR-compatible device such as nitinol microwires, allows a close monitor of the ischemia and reperfusion status and evolution. In addition, a progressive reduction of infarction volume was also noted, as previously reported in stroke patients treated with systemic thrombolysis. In fact, this phenomenon is not so rare, and DWI reversal was often sizeable in patients treated  $\leq 4.5$  hours, especially when thrombolysis was administered early.

### **Reproducibility of the infarct region**

The model characterized here seems interesting to model acute ischemic stroke in human. The presence of an ischemic penumbra after MCA occlusion makes this technique suitable for neuroprotection studies and especially ischemic postconditioning (transient cycles of ischemia conducted early before or after recanalization) [19]. However, variability of brain infarction area contributed to the lack of reproducibility of this model. The high rate (69%) of striatal infarction by perforator arteries occlusion could be explained by the technique used. In the original paper, the microcatheter was placed into the aorta [9], whereas we placed the microcatheter into the common carotid artery. This microcatheter placement opens the possibility for decreasing the cerebral blood flow, while the original technique caused infarction of the striatum in only 30.7% of cases.

As reported previously [9], our study confirms the feasibility to induce focal cerebral ischemia by selective MCA occlusion by intra-arterial approach in the rat. This method has several advantages: (1) the simplicity of the procedure; (2) the low invasiveness, as no severe disability and mortality was not observed in the postoperative period; (3) no craniotomy, and thus no ‘artificial’ alterations of intracranial pressure and brain temperature are caused; (4) the strict control of the duration of the cerebral ischaemia; (5) the high rate of intracranial arterial occlusion; (6) reperfusion is triggered intra-arterially similar to the clinical procedure in human used now.

One of the most important limitations of these models is the lack of physiopathological thrombus formation. In this context, injection of thrombin into the MCA has also been proposed as a new model of thromboembolic stroke [20]. But, invasive surgery with craniotomy is needed, and spontaneous reperfusion was observed in 80% of animals within three-hour of occlusion [21]. The endovascular approach used in this model could allow thrombus formation in the arterial lumen by thrombin injection inside the microcatheter, similarly to thromboembolic models in the rat or non-human primate [22]. The microcatheter that carries the microwire can also serve as a working channel wherein therapeutic agents or endovascular tools may be administered directly to the cerebral circulation.

In conclusion, this model of acute stroke in rats by selective intra-arterial MCA occlusion is suitable to neuroprotection studies because of possible acute and close characterization of spatiotemporal evolution of brain infarction by MR imaging techniques, and evidence of ischemic penumbra, the target of neuroprotection agents. However, optimization of the brain infarction reproducibility needs further technical and neurointerventional tools improvements.





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