A Critical Reevaluation of the Intraluminal Thread Model of Focal Cerebral Ischemia

Evidence of Inadvertent Premature Reperfusion and Subarachnoid Hemorrhage in Rats by Laser-Doppler Flowmetry

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Background and Purpose—The intraluminal thread model for middle cerebral artery occlusion (MCAO) has gained increasing acceptance. Numerous modifications have been reported in the literature, indicating that the technique has not been standardized. The present study was performed to evaluate and optimize the reliability of this model.

Methods—One hundred Sprague-Dawley rats were subjected to MCAO by 2 different intraluminal filaments. Cortical blood flow was continuously monitored over both hemispheres by laser-Doppler flowmetry (LDF). In part I (3-0 filament), we evaluated the incidence of adequate MCAO, subarachnoid hemorrhage (SAH), intraluminal thrombus formation, and the effects of heparinization. In part II (silicone-coated 4-0 filament), we also determined the influence of insufficient MCAO on morphological and functional outcome and the incidence of postischemic hyperthermia.

Results—In part I, SAH occurred in 30% and premature reperfusion in 24%. All animals with a decrease in contralateral flow had suffered SAH. Thrombus formation was not observed in any group. In part II, SAH occurred in 8% and premature reperfusion in 26%. There was no difference in outcome between rats with primary MCAO and rats with filament correction. Animals with uncorrected premature reperfusion had significantly smaller infarct volumes and fewer neurological deficits.

Conclusions—SAH and insufficient MCAO may be more common in the intraluminal thread model than previously reported. Inadvertent premature reperfusion contributes to the interanimal variability associated with this model. The incidence of valid experiments increases with the use of a silicone-coated 4-0 filament. Continuous bilateral LDF is indispensable to monitor adequate MCAO and is highly sensitive to recognize SAH.

Key Words: animal models ■ cerebral ischemia, focal ■ laser-Doppler flowmetry ■ rats

The intraluminal thread model of middle cerebral artery occlusion (MCAO) in rats, first introduced by Koizumi et al1 and later modified by Longa et al,2 has become the most widely used model to study pathophysiology and therapeutic approaches in permanent and transient focal cerebral ischemia. The model is easy to perform, minimally invasive, and does not require craniectomy, which may influence intracranial pressure, blood-brain barrier permeability, and brain temperature and may cause artifacts in imaging techniques.3,4 However, several model-inherent complications have been reported: (1) filament insertion may not result in adequate MCAO5–7; (2) inadvertent subarachnoid hemorrhage (SAH) may occur and abolish the pathophysiological relevance of the model6,8,9; (3) intraluminal thrombus formation has been reported, and some authors recommend perioperative heparinization to prevent blood clotting6,10–13; and (4) intraischemic and postischemic hyperthermia may complicate interpre-

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rinization were examined. We investigated whether laser-Doppler flowmetry (LDF) reliably indicates SAH by a decrease in contralateral local cortical blood flow (LCBF).\(^2,3\)

In the second part, the performance of a laser Doppler–guided silicone-coated 4-0 monofilament was evaluated. The incidence of insufficient MCAO, SAH, thrombus formation, and posts ischemic hyperthermia was examined. Furthermore, the influence of insufficient MCAO on the variability of morphological and functional outcome was determined.

**Materials and Methods**

One hundred male Sprague-Dawley rats weighing 270±15 g (mean±SD) were used in the present study. Animals were purchased from Charles River Laboratory (Sulzfeld, Germany). All procedures involving the animals were conducted according to institutional guidelines and were in compliance with regulations formulated by the government of Bavaria, Germany.

All animals were fasted overnight before the experiment, anesthetized in a container with 4% halothane, and administered atropine (0.5 mg/kg) subcutaneously. The rats were intubated orotracheally and mechanically ventilated with 0.8% halothane in a mixture of 70% N\(_2\)O and 30% O\(_2\) to maintain normal arterial blood gases. Temporalis muscle and rectal probes were used to monitor temperature throughout the experiment. A thermostatically regulated heating lamp and pad were used to maintain temperature at 37.0°C. The tail artery was cannulated for blood sampling and monitoring of arterial blood pressure. Serum glucose was measured before ischemia. Arterial blood gases, hemoglobin, and hematocrit were measured before, during, and after ischemia.

**Laser-Doppler Flowmetry**

LCBF was monitored in the cerebral cortex of each hemisphere in the supply territory of the middle cerebral artery (MCA) by LDF (MBF3D, Moor Instruments). Bilateral burr holes (1 mm in diameter) were drilled 5 mm lateral and 1 mm posterior to the bregma without injury to the dura mater. Then each animal was placed supine, and the head was firmly immobilized in a stereotaxic frame (model 900, David Kopf Instruments). Two rectangularly bent laser-Doppler probes (Medizin-Elektronik Lawrenz) were placed under the microscope with the aid of 2 micromanipulators and a magnifying mirror over both brain hemispheres. LCBF was continuously measured (2-Hz sampling rate) from before the onset of ischemia until 30 minutes after reperfusion. Flow values were calculated as averaged values during 1-minute periods every 10 minutes with shorter intervals immediately after induction of ischemia and reperfusion.

**Electroencephalography**

Silver electrodes were connected to the laser-Doppler probes for continuous electroencephalographic (EEG) recording (EEG-7109, Nihon Kohden Kogyo Ltd) from the cortex of both hemispheres with a reference electrode over the lower jawbone. Band pass was set at 0.15 to 45 Hz and amplitude at 1.2 mm/50 \(\mu\)V.

**Part I**

Fifty rats were randomly assigned to 1 of 2 groups (n=25 each) receiving either intra-arterial injections of heparin (150 IU/kg) 15 minutes before and 1 hour after insertion of the filament or the same volume of normal saline. All rats were subjected to MCAO by insertion of a 3-0 surgical nylon monofilament (Ethicon), and no heparin was administered. The silicone filament was prepared as recommended by M.-L. Smith, PhD, and B.K. Siesjö, MD, PhD (personal communication). Briefly, the filament was introduced into a polyethylene catheter with 0.28-mm inner diameter (Portex Co), which was then filled with silicone (Rhodorsil RTV 1556 A and B Pink). The polyethylene catheter was removed after hardening of the silicone. The filament was advanced until ipsilateral flow decreased to ~20% of baseline, as described in part I. If ipsilateral LDF indicated premature reperfusion, the filament was immediately corrected in every second animal. In the other half of the animals with a steep increase in ipsilateral laser-Doppler signal, the position of the filament was not corrected. After 90 minutes the filament was withdrawn into the stump of the ECA. Thirty minutes after reperfusion anesthesia was discontinued, and animals were allowed to recover.

As in part I, temporalis muscle temperature and rectal temperature were maintained at 37°C during anesthesia. In the awake animals the rectal temperature was measured 1 hour after extubation and then daily during the 7-day observation period. Neurological deficits of the animals were evaluated daily by a “blinded” coworker using a 6-point neurological function score that was modified after that described by Bederson et al:\(^4\): 0, no spontaneous activity; 1, spontaneous circling; 2, circling if pulled by tail; 3, lowered resistance to lateral push without circling; 4, contralateral forelimb flexion; and 5, no apparent deficit.

Seven days after ischemia, the rats were anesthetized by chloral hydrate and perfused transcardially by 2% paraformaldehyde. The brains were removed, embedded in paraffin, and cut into 4-μm-thick coronal sections at 400-μm intervals. The brain slices were stained with hematoxylin and eosin and Ladewig’s trichrome stain for detection of fibrin. All slices were assessed for signs of SAH and intraluminal thrombus formation.

**Part II**

Fifty male Sprague-Dawley rats were subjected to the same operative procedure as in part I, except that MCAO was performed by introducing a silicone-coated 4-0 nylon monofilament (Ethicon), and no heparin was administered. The silicone filament was prepared as recommended by M.-L. Smith, PhD, and B.K. Siesjö, MD, PhD (personal communication). Briefly, the filament was introduced into a polyethylene catheter with 0.28-mm inner diameter (Portex Co), which was then filled with silicone (Rhodorsil RTV 1556 A and B Pink). The polyethylene catheter was removed after hardening of the silicone. The filament was advanced until ipsilateral flow decreased to ~20% of baseline, as described in part I. If ipsilateral LDF indicated premature reperfusion, the filament was immediately corrected in every second animal. In the other half of the animals with a steep increase in ipsilateral laser-Doppler signal, the position of the filament was not corrected. After 90 minutes the filament was withdrawn into the stump of the ECA. Thirty minutes after reperfusion anesthesia was discontinued, and animals were allowed to recover.

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Infarct volume was calculated by multiplying the infarct area of each brain by a blinded examiner. Statistical analysis was performed with the use of SigmaStat 2.0 (Jandel Scientific). Parametric data were analyzed with 1-way ANOVA and neurological function scores with Kruskal-Wallis ANOVA on ranks for each of the 7 days. If multiple comparisons were indicated, the Bonferroni’s t-test for normally distributed data and the nonparametric Dunnell’s t-test for neurological function scores were applied. The Dunnell’s t-test was used when the sample size varied. Significance was accepted at the \(P<0.05\) level. Results are presented as mean±SD.

**Results**

**Physiological Variables**

All physiological variables remained within normal limits. The experimental groups did not differ with respect to attention was paid if there was a perceptible resistance before the ipsilateral flow decreased. The filament was secured at the ECA by a tight ligature and a microclip. If the ipsilateral laser-Doppler signal showed a steep increase in blood flow during the occlusion period, premature reperfusion was suspected and the filament was readjusted. After 90 minutes the filament was withdrawn into the stump of the ECA to allow reperfusion. Thirty minutes after reperfusion all animals were killed by transcardiac perfusion with 2% paraformaldehyde. The brains were removed, embedded in paraffin, and cut into 4-μm-thick coronal sections at 400-μm intervals. The brain slices were stained with hematoxylin and eosin and Ladewig’s trichrome stain for detection of fibrin. All slices were assessed for signs of SAH and intraluminal thrombus formation.

**Statistical Analysis**

Statistical analysis was performed with the use of SigmaStat 2.0 (Jandel Scientific). Parametric data were analyzed with 1-way ANOVA and neurological function scores with Kruskal-Wallis ANOVA on ranks for each of the 7 days. If multiple comparisons were indicated, the Dunnell’s t-test for normally distributed data and the nonparametric Dunnell’s t-test for neurological function scores were applied. The Dunnell’s t-test was used when the sample size varied. Significance was accepted at the \(P<0.05\) level. Results are presented as mean±SD.
preischemic, intraischemic, or postischemic blood pressure or arterial blood gases. There were no significant differences in hemoglobin, hematocrit, or serum glucose between the groups.

During anesthesia temporalis muscle and rectal temperature were maintained at 37.0°C. Intraoperative cooling was not necessary. In all surviving animals rectal temperature remained in the range between 37.0°C to 38.5°C (37.7±0.5°C; mean±SD) during the 7-day observation period. Postischemic hyperthermia of $39.0°C$, which was reported to occur in the intraluminal thread model by another group,14,15 was not observed in our study.

Part I

Laser-Doppler Flowmetry

The 3-0 filament was advanced until ipsilateral LCBF decreased in all 50 rats. In 11 (of 50) animals a resistance was perceptible while the filament was placed, but LDF indicated lack of MCAO. The filament was further advanced until the ipsilateral laser-Doppler signal decreased. In 12 (of 50) rats, ipsilateral LDF indicated premature reperfusion at various times, usually during the first 15 minutes after induction of ischemia. The filament was immediately readjusted in all 12 rats to reduce ipsilateral blood flow to 20% to 30% of baseline.

In 28 (of 50) rats contralateral LCBF remained stable throughout the experiment, fluctuating at $\approx 100$% of baseline. In these rats withdrawal of the filament after 90 minutes was followed by initial hyperemia and then by a gradual decrease in ipsilateral blood flow to $\approx 70$% of baseline. Delayed hypoperfusion persisted until the end of the recording period at 30 minutes after reperfusion. None of these animals had signs of SAH, as demonstrated later by histopathology. There were no significant differences in LCBF between nonheparinized and heparinized rats with stable contralateral LCBF (Figure 1A and 1B).

In 22 (of 50) rats contralateral LCBF significantly decreased immediately after filament placement (n=15) or readjustment (n=7). In 11 (of 25) nonheparinized and in 11 (of 25) heparinized rats contralateral LCBF fell to 48±34% and 53±37% (mean±SD) of baseline, respectively. Contralateral LCBF never recovered to baseline in these animals. After filament withdrawal, ipsilateral LDF indicated lack of reperfusion in all 22 rats with a decrease in contralateral LCBF after filament placement. All these rats had suffered SAH, as demonstrated later by histopathology (Figure 1C and

Figure 1. Dynamic changes in ipsilateral ($A$) and contralateral ($C$) LCBF measured by LDF. Arrows indicate insertion and withdrawal of the filament. Values are mean±SD. $A$, Nonheparinized rats without SAH (n=14). $B$, Heparinized rats without SAH (n=14). $C$, Nonheparinized rats with SAH (n=11). $D$, Heparinized rats with SAH (n=11). There was no significant difference between nonheparinized and heparinized rats without SAH (A vs B) ($P>0.05$, 1-way ANOVA followed by Dunn’s test for all pairwise comparisons for each time point). The decrease in contralateral flow immediately after filament insertion in rats with SAH (C and D) was highly significant ($P<0.01$) compared with rats without SAH, which showed normal contralateral flow around baseline (A and B). The decrease in contralateral LCBF after filament withdrawal in heparinized rats with SAH (D) was more pronounced ($P<0.05$) compared with nonheparinized rats with SAH (C).
The decrease in contralateral flow immediately after filament insertion in rats with SAH was highly significant \( (P<0.01) \) compared with rats without SAH, which showed normal contralateral flow around baseline. The decrease in contralateral LCBF after filament withdrawal in heparinized rats with SAH \( (13\pm9\% \text{ of baseline}) \) was more pronounced \( (P<0.05) \) than in nonheparinized rats with SAH \( (42\pm34\% \text{ of baseline}) \) (1-way ANOVA followed by Dunn’s test for all pairwise comparisons for each time point).

**Electroencephalography**

EEG was used qualitatively to reveal whether a successful MCAO and reperfusion as indicated by LDF had been achieved. MCAO caused severe suppression of the ipsilateral hemispheric record. Contralateral suppression of the EEG was observed when LDF indicated a decrease in contralateral LCBF. EEG suppression occurred with a delay of 5 to 10 seconds after the decrease in LDF. However, EEG did not indicate premature reperfusion as shown by LDF and did not recover after reperfusion during the recording period.

**Histopathology**

In animals with an initial decrease in contralateral LCBF, histopathology always revealed SAH. SAH was absent in rats with normal contralateral LCBF around baseline. Heparinized rats suffered a more intensive hemorrhage with larger subarachnoid and intraventricular blood clots. No signs of intraluminal thrombus formation were seen in either group.

The results of part I as demonstrated by LDF and histology are summarized in Table 1. A perceptible resistance when the filament was advanced did not reliably indicate adequate MCAO. Overall, MCAO was achieved in 56\% of cases: in 46\% by primary MCAO and in 10\% after filament correction. SAH occurred in 44\% of the cases: in 30\% during initial filament placement and in 14\% during filament correction. LDF was highly sensitive (100\%) for SAH.

**Part II**

**Laser-Doppler Flowmetry**

The silicone-coated 4-0 filament was advanced until the ipsilateral laser-Doppler signal decreased. In 33 (of 50) rats primary MCAO was achieved, but a resistance was felt in only 12 of these 33 rats. As in part I, MCAO caused an immediate decrease in ipsilateral LCBF in the MCA territory to 20\% to 30\% of baseline. Contralateral LCBF remained unchanged and fluctuated at \( \approx 100\% \text{ of baseline} \) in these animals. After filament withdrawal, initial postischemic hyperemia was followed by a gradual decrease in blood flow to \( \approx 70\% \text{ of baseline} \). Delayed hypoperfusion persisted until the end of the recording period at 30 minutes after reperfusion.

In 4 (of 50) rats a decrease in contralateral LCBF was observed during initial placement of the filament. In 3 of the 4 rats a resistance was perceptible, but LDF indicated lack of MCAO. When the filament was further advanced, ipsilateral and contralateral LCBF decreased. These rats were excluded from the study, and SAH was confirmed by autopsy.

In 13 (of 50) rats a steep increase in ipsilateral laser-Doppler signal indicated premature reperfusion at various times after filament placement, usually during the first 15 minutes, but premature reperfusion occurred as late as 60 minutes after induction of ischemia. A resistance was perceptible in 4 of these 13 rats.

The filament was readjusted in every second animal with premature reperfusion. The filament was readjusted in 7 (of 13) animals, which caused a decrease in contralateral LCBF in 2 rats. These 2 rats were excluded from the study, and SAH was confirmed by autopsy. In the 6 (of 13) rats in which the filament was not readjusted, despite that fact that LDF indicated premature reperfusion, various laser-Doppler courses from single peaks to full recovery of LCBF to baseline were observed. Exemplary laser-Doppler courses of premature reperfusion without and with correction of the filament are presented in Figure 2.

The results of part II are summarized in Table 2. In 33 rats definite MCAO was achieved by primary filament placement, and in 5 rats definite MCAO was achieved after filament correction. Six rats suffered SAH: 4 rats during primary filament placement and 2 rats during filament correction. In 6 rats with premature reperfusion, the filament was not corrected. Overall, definite MCAO (by primary MCAO or after filament correction) was attempted in 44 rats and achieved in 38 rats (86\%).

**Electroencephalography**

As in part I, EEG suppression occurred with a delay of 5 to 10 seconds after the decrease in LCBF as shown by LDF. Again, EEG did not indicate premature reperfusion and did not recover after reperfusion during the recording period.

**Neurological Deficits**

There was no statistically significant difference in functional outcome between rats with primary MCAO and rats with secondary MCAO in which the filament was immediately repositioned. All rats with MCAO postoperatively exhibited a
Figure 2. Exemplary screen copies of laser-Doppler courses in individual rats subjected to 90 minutes of MCA occlusion and reperfusion. The short drop in ipsilateral flow before ischemia is due to transient clipping of the CCA for insertion of the occluding filament. Insertion and withdrawal of the filament are marked by vertical lines. A, Normal laser-Doppler course with 90 minutes of ischemia followed by initial peak hyperemia and delayed hypoperfusion after filament withdrawal. B, Premature reperfusion without filament correction. In this example intermittent reperfusion occurred ~25 minutes after induction of ischemia. C, Premature reperfusion with immediate filament correction ~8 minutes after induction of ischemia. D, SAH indicated by a simultaneous decrease in ipsilateral and contralateral flow after insertion of the filament and a second decrease instead of reperfusion after filament withdrawal.
contralateral hemiparesis and recovered during the observation period. There was no mortality.

In contrast, rats with uncorrected premature reperfusion had significantly fewer neurological deficits than rats with primary MCAO and rats in which the filament was repositioned (P<0.05, Kruskal-Wallis test followed by nonparametric Dunn’s test for each of the 7 postoperative days). Two animals had no neurological deficits, but 4 rats showed signs of contralateral hemiparesis. The neurological function scores on postoperative days 1 and 7 are presented in Table 2.

**Histopathology**

Infarct volumes (mean±SD) are presented in Table 2. There was no statistically significant difference in infarct volume between rats with primary MCAO (65±19 mm³) and rats with secondary MCAO in which the filament was immediately repositioned (64±18 mm³). Infarct volume in rats with uncorrected premature reperfusion (39±30 mm³) was significantly smaller than in the 2 other groups (P<0.05, 1-way ANOVA followed by Dunn’s test for all pairwise comparisons). Animals with a decrease in contralateral LCBF were excluded from the study, and SAH was confirmed by autopsy. In all other rats there were no histological signs of SAH or intraluminal thrombus formation.

**Discussion**

This study was performed to further standardize the intraluminal thread model and to optimize its reproducibility. Insufficient MCAO and inadvertent SAH are the most common complications of this model; they can be overcome by the use of continuous bilateral LDF and a silicone-coated 4-0 filament. MCAO was achieved in a much higher proportion (56%) of the experiments, and the incidence of SAH was much less frequent (25%) of the experiments. The filament may be inserted through the ECA, ICA, or CCA.1,2,5,11,19,28 The latter 2 methods result in a permanent occlusion of the ipsilateral carotid artery. Tandem occlusion of the MCA and ipsilateral or bilateral CCA reduces cerebral blood flow (CBF) in the core and periphery of the MCA territory and has been reported to produce more consistent infarct volumes.29–33 Nevertheless, we chose to occlude the MCA through the ECA because permanent CCA occlusion limits reperfusion.3,4

As demonstrated in part II of the experiments, premature reperfusion can be recognized by continuous LDF and be corrected without influencing the outcome.

Since the first description by Koizumi et al.,1 numerous investigators performed modifications of the intraluminal thread model concerning (1) the technique of filament insertion, (2) duration of MCAO with or without simultaneous ipsilateral or bilateral CCA occlusion, (3) different filaments with their tip rounded by heat or sandpaper or covered by silicone or poly-L-lysine, (4) strain and body weight of the rats, (5) heparinization to prevent blood clotting, and (6) other technical factors to optimize reproducibility of this model.*

**Technique of Filament Placement**

The filament may be inserted through the ECA, ICA, or CCA.1,2,5,11,19,28 The latter 2 methods result in a permanent occlusion of the ipsilateral carotid artery. Tandem occlusion of the MCA and ipsilateral or bilateral CCA reduces cerebral blood flow (CBF) in the core and periphery of the MCA territory and has been reported to produce more consistent infarct volumes.29–33 Nevertheless, we chose to occlude the MCA through the ECA because permanent CCA occlusion limits reperfusion.3,4

With laser Doppler–guided filament placement, adequate MCAO could be achieved in 56% (uncoated 3-0 filament) and 86% (silicone-coated 4-0 filament) of the experiments. Several methods have been described in the literature regarding how to place the filament and block the origin of the MCA.2,5,11

The first method involves advancing the filament until a faint resistance is felt after passing the skull base.2,5 According to our experience, the surgeon’s ability to notice the resistance increases with practice, but there is not always a resistance. LDF in the present study indicated that vessel perforation may occur before any resistance is perceptible. On the other hand, there may be a clear resistance although the MCA is still not occluded, and further advancement commonly leads to SAH.

*References 2, 6–8, 11, 13, 16, 17, 19, 21, 22, 26, 27.

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**TABLE 2. Part II: Summary of Results**

<table>
<thead>
<tr>
<th></th>
<th>No. of Animals</th>
<th>Postoperative Day</th>
<th>Neurologic Score</th>
<th>Infarct Volume, mm³ (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Resistance</td>
<td>Percentage</td>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>Primary MCAO</td>
<td>33 (12)</td>
<td>66</td>
<td>Day 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
<td>3</td>
</tr>
<tr>
<td>Primary SAH</td>
<td>4 (3)</td>
<td>8</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>Premature reperfusion</td>
<td>13 (4)</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With filament correction</td>
<td>7 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary MCAO</td>
<td>5 (3)</td>
<td></td>
<td>Day 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
<td>3</td>
</tr>
<tr>
<td>Secondary SAH</td>
<td>2 (1)</td>
<td>Excluded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without filament correction</td>
<td>6 (NA)</td>
<td>Day 1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
<td>5</td>
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References 2, 6–8, 11, 13, 16, 17, 19, 21, 22, 26, 27.
The second method involves advancing the filament for a defined length. In a series of experiments to confirm the optimal surgical technique, Longa et al. reported that MCAO was standardized by advancing the filament exactly 17 mm into the ICA from the origin of the ECA in Sprague-Dawley rats weighing 300 to 400 g. Other groups advance the filament as far as 22 mm in Sprague-Dawley rats weighing 280 to 340 g. Garcia et al.33,35 stated that a close relationship exists between the animal’s body weight and the length of the filament required to reach the origin of the anterior cerebral artery with the tip of the filament. This group used Wistar rats weighing 270±15 g and inserted the filament 18.5±0.2 mm. Zarow et al.6 compared the effect of filament insertion distance on CBF, neurological deficits, and infarct volume in Sprague-Dawley rats weighing 280 to 320 g. They found that blood flow reduction, neurological deficits, and ischemic damage after permanent MCAO were more severe and more reliably produced when the filament was advanced 22 mm distal to the CCA bifurcation than when it was advanced only 18 mm. Evidently, these guidelines must be determined for each strain and weight class.21,37

Third, Memezawa et al.11 judged successful MCAO by unilateral EEG suppression. The proportion of rats that failed to show the expected changes in EEG and neurological behavior was 17% in their study. We found that EEG reliably indicates MCAO by ipsilateral and SAH by bilateral hemispheric suppression with a delay of 5 to 10 seconds compared with the laser-Doppler signal. This delay makes adequate filament placement more difficult. Furthermore, EEG does not indicate premature reperfusion during ischemia, nor does it indicate reperfusion after filament withdrawal.

Without continuous bilateral LDF, it remains difficult to judge whether the MCA was adequately occluded or whether SAH had occurred. Because of this uncertainty, some investigators exclude 20% to 30% of the animals from their studies.36,39 Animals with no or only minor neurological deficits are excluded with the presumption that MCAO was insufficient.11,38–41 Animals with severe neurological deficits are suspected to have suffered SAH and are also excluded.38–40 This practice may obscure both neuroprotective and adverse effects when drugs are tested.

Effects of Filament Properties on Adequate MCAO

The incidence of primary SAH when a 3-0 filament with its tip rounded by heat was used in part I was reduced from 30% to 8% when a silicone-coated 4-0 filament was used in part II of the experiments. The high rate of SAH in part I is explained by (1) the stiffness and tip of the 3-0 filament, (2) the fact that the filament was further advanced beyond a perceptible resistance when LDF indicated lack of MCAO, and (3) the fact that all animals were examined for signs of SAH. In our experience, the 3-0 filament with its tip rounded by heat perforates the vessel wall more easily without any perceptible resistance compared with the more flexible silicone-coated 4-0 filament. Consistent with our results, Jakubowski et al. stated that the use of a 3-0 filament is a very effective means of producing SAH in rats, and Takano et al. suggested that coating a 4-0 filament with a thin layer of low-viscosity silicone prevents this complication. Laing et al. compared the performance of an uncoated 4-0 filament with its tip rounded by heat (the method of Longa et al.) and of a silicone-coated 4-0 filament (the method of Koizumi et al.). They found that by the method of Koizumi et al. MCAO is achieved in a much higher proportion of cases (93%), and the incidence of perforation of the intracranial carotid artery is much less frequent than by the method of Longa et al., in which MCAO was achieved in only 56% of the cases. The depth of ischemia was more profound with the filament of Koizumi et al. (9 mL/100 g per minute) than with the filament of Longa et al. (36 mL/100 g per minute). The authors attributed this to residual blood flow around the uncoated and therefore thinner 4-0 filament. This variation in the resultant CBF with the use of this model emphasizes the need to determine CBF during the occlusion and reperfusion phase.37 The diameter and quality of a thread seem to be very important for the reproducibility and reliability of MCAO.35 Kuge et al. have shown that exact diameter and quality are not always the same among nylon monofilaments, even if they meet the standard for the designation 4-0. They demonstrated that slight differences of filament characteristics significantly affect lesion volume and reproducibility. Belayev et al. coated a 3-0 filament with poly-l-lysine and compared its performance with an uncoated 3-0 filament. Poly-l-lysine molecules absorb strongly to solid surfaces and may encourage adhesion of the filament to the adjacent vascular endothelium without changing the diameter of the filament. They reported that the poly-l-lysine-coated filament produced consistently larger infarcts and greatly reduced interanimal variability.5,44

Premature Reperfusion

Premature reperfusion requiring readjustment of the filament occurred in ≈25% of the experiments, irrespective of the kind of filament used. If experiments with primary SAH are excluded, premature reperfusion may complicate up to one third of the remaining experiments. Since premature reperfusion usually occurred during the first minutes after MCAO, we assume that the concomitant rise in arterial blood pressure causes a slight dislocation of the filament. In part II of the study, we evaluated differences in outcome after primary MCAO and premature reperfusion with and without readjustment of the filament. As shown in Table 2, immediate readjustment of the filament provides consistent results with primary MCAO. If the filament is immediately corrected, the reperfusion period is too short and incomplete (Figure 2C) to influence outcome, as reported with intermittent reperfusion periods of 5 to 15 minutes’ duration.45,46 However, unnoticed filament dislocation (Figure 2B) may contribute to the high variability of this model.

To our knowledge there are no reports about premature reperfusion after initial correct placement of the filament. Dislocation of the filament is not visible under the operating microscope. EEG does not indicate premature reperfusion, and experiments controlled by continuous LDF without repositioning the probes are scarce.12,21 Obtaining a baseline measurement of cortical flow before ischemia and repositioning of the laser-Doppler probes afterward have been proven to provide unreliable results.47 We did not evaluate the hydrogen clearance technique with regard to premature reper-
fusion, but we expect a lower sensitivity because of its limited temporal resolution compared with LDF.12,46,48,49

**Heparinization and Thrombus Formation**

Another concern with the intraluminal thread model is that the occluder may cause endothelial damage to the vessel wall and that thrombosis occurs during or after MCAO.1,7,13 Some groups therefore use heparin to avoid blood clotting.11,46,50 In our experiments neither nonheparinized nor heparinized rats showed any sign of thrombus formation. Heparin did not influence LCBF except in rats with SAH. As expected, heparin did not increase the incidence of SAH but was associated with a more pronounced rebleeding after withdrawal of the filament. Rabb13 found that approximately 15% to 20% of rats sustain a very large infarct as a result of thrombosis and subsequent permanent MCAO despite systemic heparinization during the procedure. Kawamura et al19 observed thrombus formation in the ICA only when both the MCA and the pterygopalatine artery were occluded permanently. They stated that thrombus formation does not occur when the pterygopalatine artery is kept patent. We rarely encountered insurmountable difficulties in passing the pterygopalatine artery and therefore never occluded it. Clearly, the effects of different intraluminal filaments on the endothelium and coagulation system need further investigation.

**Hyperthermia**

We did not observe intraoperative or postoperative hyperthermia, as was recently reported to occur in the intraluminal thread model.14,15 Zhao et al13 and Memezawa et al14 speculated that hyperthermia may be caused by hypothalamic ischemia and demonstrated that it nullifies therapeutic effects of MK-801 in hyperthermia may be caused by hypothalamic ischemia and that thrombosis occurs during or after MCAO.1,7,13 Some groups therefore use heparin to avoid blood clotting.11,46,50 In our experiments neither nonheparinized nor heparinized rats showed any sign of thrombus formation. Heparin did not influence LCBF except in rats with SAH. As expected, heparin did not increase the incidence of SAH but was associated with a more pronounced rebleeding after withdrawal of the filament. Rabb13 found that approximately 15% to 20% of rats sustain a very large infarct as a result of thrombosis and subsequent permanent MCAO despite systemic heparinization during the procedure. Kawamura et al19 observed thrombus formation in the ICA only when both the MCA and the pterygopalatine artery were occluded permanently. They stated that thrombus formation does not occur when the pterygopalatine artery is kept patent. We rarely encountered insurmountable difficulties in passing the pterygopalatine artery and therefore never occluded it. Clearly, the effects of different intraluminal filaments on the endothelium and coagulation system need further investigation.

In summary, this popular model involves several pitfalls that can be overcome by the use of continuous bilateral LDF. Meticulous attention should be paid to various aspects, including inadvertent SAH and insufficient CBF reduction, intraluminal thrombus formation, postischemic hyperthermia, and the operative technique. According to our results, thrombus formation does not seem to be a major problem, and postischemic hyperthermia does not occur after isolated MCAO in Sprague-Dawley rats when duration of ischemia is limited to 90 minutes. However, SAH and insufficient MCAO may be more common in the intraluminal thread model than previously reported. The chance of valid experiments increases with the use of a silicone-coated 4-0 filament. Continuous bilateral LDF is indispensable to reliably achieve and monitor adequate MCAO and is highly sensitive (100%) to recognize SAH during the operation, thus saving time and expense.

**References**


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The method to occlude in rats one MCA by insertion of a nylon monofilament through the external carotid artery has been widely adopted by many researchers, as indicated by the increasing number of publications dealing with either modifications (ie, improvements) to the original technique or applications of the “thread method” to experiments that aim to increase our understanding of specific events initiated by the arterial occlusion.

The meticulous analysis of 100 experiments completed at the prestigious research laboratory of Ludwig Maximilians University (Munich, Germany) provides useful additional information on the reliability of the method based on the use of a nylon filament to occlude the MCA in Sprague-Dawley rats.

Schmid-Elsaesser and colleagues report in this study 5 main items: (1) Significant variations in body temperature (hyperthermia) were not observed during surgery or in the postoperative period (7 days). This is in contrast to results reported from other equally prestigious laboratories. (2) Repositioning the filament in rats in which the laser-Doppler flowmetry indicated “reperfusion” to the cortex led to a secondary stage of ischemia. Use of a silicone-coated filament (4-0) increased the rate of success in inducing primary or persistent regional ischemia. (3) Development of ischemia in the cortex of the contralateral hemisphere (as indicated by laser-Doppler flowmetry) presaged the development of subarachnoid hemorrhage. (4) Intraluminal thrombus formation in the MCA or its main branches was not detectable in any of the rats, and heparinized rats developed larger hemorrhages (subarachnoid and intraventricular) compared with those not given heparin. (5) The volume of the brain infarct produced in these experiments, based on 90-minute MCA occlusion and 7-day reperfusion, was comparable in the groups with either “primary” or “secondary” MCA occlusion.

The authors deserve high commendation for their contributions to widen the application of this useful model to experiments in which we will continue to expand our understanding of ischemic strokes.

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A Critical Reevaluation of the Intraluminal Thread Model of Focal Cerebral Ischemia: Evidence of Inadvertent Premature Reperfusion and Subarachnoid Hemorrhage in Rats by Laser-Doppler Flowmetry

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