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 model5,8,9; (3) intraluminal thrombus formation has been reported, and some authors recommend perioperative heparinization to prevent blood clotting1,10–13; and (4) intraschismic and postischemic hyperthermia may complicate interpretation of the results.14,15 These complications may be responsible for the considerable variability of the extent of ischemic neuronal injury.5,8,13,16–18 The numerous modifications reported in the literature also indicate that the model has not yet been standardized.1,2,7,11,19–22

The aim of the present study was to better standardize this model and optimize its reliability. We therefore examined the following questions: (1) How may MCAO be obtained and monitored reliably? (2) How may the incidence of SAH be reduced and its occurrence recognized early? (3) Does intraluminal thrombus formation occur, and is heparinization necessary? (4) Is postischemic hyperthermia a frequent complication?

In the first part of our experiments the performance of a 3-0 monofilament was evaluated. The incidence of insufficient MCAO, SAH, thrombus formation, and the effects of hepa-
inzination were examined. We investigated whether laser-Doppler flowmetry (LDF) reliably indicates SAH by a decrease in contralateral local cortical blood flow (LCBF).9,23

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 postischemic 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% N2O and 30% O2 to maintain normal arterial blood gases. Rectal temperature was measured before and 1 hour after insertion of the filament or the same saline. 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.

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 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 al24: 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 paraaffin, 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. The infarct areas were assessed planimetrically (OPTIMAS 5.1, BioScan Inc) by a blinded examiner. For each brain, 24 slices were measured encompassing the entire infarct. Only areas of pan necrosis consisting of the loss of affinity for hematoxylin that affects all cell types (neuronal, glial, and vascular) were measured. Infarct volume was calculated by multiplying the infarct area of each slice by the distance (400 μm) between successive slices.

Statistical Analysis

Statistical analysis was performed with the use of SigmaStat 2.0 Statistical Software (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 Dunnett’s test for normally distributed data and the nonparametric Dunnett’s test for neurological function scores were applied. The Dunn’s 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...
preischemic, intraischemic, or posts ischemic 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. Posts ischemic 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 ~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 ~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 1D).

Figure 1. Dynamic changes in ipsilateral (F) and contralateral (E) 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 ($13\pm9\%$ of baseline) was more pronounced ($P<0.05$) than in nonheparinized rats with SAH ($42\pm34\%$ 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 the 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 intraluminal thrombus formation were seen in either group. Filament repositioning was attempted in 44 rats and achieved in 36 rats with premature reperfusion without and with correction of the filament were presented in Figure 2.

The results of part II are summarized in Table 2. In 33 rats defined MCAO was achieved by primary filament placement, and in 5 rats defined MCAO was achieved after filament correction. Six rats suffered SAH: 4 rats during primary filament placement and 2 rats during filament correction. In 5 rats with premature reperfusion, the filament was not corrected. Overall, definite MCAO (by primary MCAO or after filament correction) was attempted in 46 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 LDF 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

### Table 1. Part I: Summary of Results

<table>
<thead>
<tr>
<th></th>
<th>Nonheparinized (n=25)</th>
<th>Heparinized (n=25)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary MCAO</td>
<td>12 (4)</td>
<td>11 (3)</td>
<td>46</td>
</tr>
<tr>
<td>Primary SAH</td>
<td>8 (2)</td>
<td>7 (2)</td>
<td>30</td>
</tr>
<tr>
<td>Premature reperfusion and filament correction</td>
<td>5 (2)</td>
<td>7 (2)</td>
<td>24</td>
</tr>
<tr>
<td>Secondary MCAO</td>
<td>2 (1)</td>
<td>3 (0)</td>
<td>10</td>
</tr>
<tr>
<td>Secondary SAH</td>
<td>3 (1)</td>
<td>4 (2)</td>
<td>14</td>
</tr>
<tr>
<td>Intraluminal thrombus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
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.
TABLE 2. Part II: Summary of Results

<table>
<thead>
<tr>
<th>No. of Animals (Resistance Perceptible)</th>
<th>Percentage</th>
<th>Postoperative Day</th>
<th>Neurologic Score</th>
<th>Infarct Volume, mm³ (Mean±SD)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median 25% Percentile 75% Percentile Range</td>
<td></td>
</tr>
<tr>
<td>Primary MCAO</td>
<td>33 (12)</td>
<td>66</td>
<td>Day 1</td>
<td>2 1 3 1–4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
<td>3 2 4 2–5</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 1.75 2.25 1–3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
<td>3 2 4.25 2–5</td>
</tr>
<tr>
<td>Secondary SAH</td>
<td>2 (1)</td>
<td></td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>Without filament correction</td>
<td>6 (NA)</td>
<td></td>
<td>Day 1</td>
<td>4 4 5 3–5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
<td>5 5 5 4–5</td>
</tr>
</tbody>
</table>

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 significantly smaller than in the 2 other groups (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.

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 of cases, and the incidence of SAH was much less frequent with laser Doppler–guided placement of a 4-0 silicone-coated filament. Continuous LDF of the contralateral brain hemisphere offers a highly reliable method of early detection of SAH. It is possible to recognize such a course, which is likely to adversely influence the outcome from the experiment, during filament placement.

Furthermore, we found that a hitherto unreported premature reperfusion may occur in ~25% of the experiments, irrespective of the kind of filament used. This premature reperfusion may contribute to the high variability in infarct size and neurological outcome associated with this model.5,8,25 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.1,2,7,11,19,28 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.6,34

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.2,7 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.
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. 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. 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.

Third, Memezawa et al. 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. Animals with no or only minor neurological deficits are excluded with the presumption that MCAO was insufficient. Animals with severe neurological deficits are suspected to have suffered SAH and are also excluded. 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 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. The diameter and quality of a thread seem to be very important for the reproducibility and reliability of MCAO. 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.

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. 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. Obtaining a baseline measurement of cortical flow before ischemia and repositioning of the laser-Doppler probes afterward have been proven to provide unreliable results. 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. 5,46,48,49

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. 13,15 Zhao et al15 and Memezawa et al14 speculated that hyperthermia may be caused by hypothalamic ischemia and demonstrated that it nullifies therapeutic effects of MK-801 in rats. 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, posts ischemic hyperthermia, and the operative technique. According to our results, thrombus formation does not seem to be a major problem, and posts ischemic 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.

Acknowledgments

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References

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 method 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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