A new method for superselective middle cerebral artery infusion in the rat

JOHANNES WOITZIK, M.D., AND LOTHAR SCHILLING M.D., PH.D.
Department of Neurosurgery, University Hospital Mannheim, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, Mannheim, Germany

Object. Selective intraarterial drug delivery is used to achieve enhanced local uptake with reduced systemic side effects. In the present paper the authors describe and characterize a new microcatheter-based model of superselective perfusion of the middle cerebral artery (MCA) in rats combined with blockade of blood flow through the MCA.

Methods. Selectivity of administration was shown by infusion of Evans blue which diffusely stained the MCA territory, indicating an increased permeability of the blood–brain barrier during the blockade of blood flow to the MCA. Perfusion of autologous blood through the microcatheter resulted in a flow rate–related increase in the cerebral blood flow measured by laser Doppler flowmetry. Similarly, infusion of an artificial O₂ carrier, Oxycyte, was accompanied by an increase in tissue oxygenation as measured using a Licox sensor. Blockade of blood flow to the MCA with the new microcatheter for an extended period of time resulted in the development of ischemia, which was comparable to that induced by intravascular occlusion using a silicone-coated thread. In a 24-hour MCA occlusion model, selective administration of a low dose of MK-801 (0.3 mg/kg body weight) resulted in a significantly smaller infarct volume than systemic application (339 ± 53 mm³ compared with 508 ± 26 mm³, p < 0.001).

Conclusions. This new model of superselective MCA infusion is a valuable tool for investigating the effect of selective delivery and enhanced drug uptake into cerebral ischemic tissue. Without constant blockade of blood flow through the MCA it may also be useful for enhanced drug uptake, gene transfer, or application of stem cells in other neuropathological conditions.

Key Words • cerebral blood flow • focal ischemia • blood–brain barrier • drug delivery • laser Doppler flowmetry • rat

Selective intraarterial drug injection results in high local drug concentrations and reduced systemic side effects compared with systemic administration. In the cerebrovascular system intraarterial drug application is used for various conditions that affect the brain such as plasminogen activators for thrombolysis in embolic stroke disease, papaverine or nicardipine for relief of vasospasm after subarachnoid hemorrhage, or enhanced chemotherapy delivery in malignant gliomas. However, the BBB allows only limited access for many substances despite a high intravascular concentration during local delivery. Osmotic disruption or pharmacological modification of the BBB has previously been used to increase drug uptake, although with limited success in the clinical setting.

One of the most serious threats to the functional integrity of the BBB is posed by hypoxia and ischemia. Selective catheterization of individual arteries easily allows vessel occlusion and induction of local hypoxia or ischemia in the tissue downstream of the occlusion. Thus, selective catheterization and temporary occlusion of a cerebral artery could be used to achieve improved drug delivery and enhanced uptake into brain tissue. However, there are only a few animal models available for experimental analysis, and catheterization and selective infusion into a cerebral artery have usually been performed in large animal species. We have, therefore, developed a new catheter device for superselective and highly controllable MCA perfusion in rats. Selectivity and control of perfusion are brought about by blocking blood flow through the MCA from both the anterior and posterior supply from the circle of Willis, resulting in focal hypoxia or even ischemia upon extended positioning of the catheter. The selectivity of the approach is proven by dye infusion. The modulation of physiological parameters including blood flow and tissue oxygenation is shown by administration of blood and an artificial O₂ carrier, respectively. The therapeutic promise of this new approach is demonstrated by a short-term infusion of MK-801, a putative neuroprotectant in a model of permanent focal ischemia.

Materials and Methods

Catheter Design
A monofile 6-0 polyamide suture (Ethicon, Johnson & Johnson)
Selective MCA catheterization in rats

was heat blunted and subsequently coated with epoxide glue to give a tip diameter of 370 to 380 μm. A 30-mm-long polyethylene microtube (inner diameter 0.28 mm, outer diameter 0.61 mm; Portex) was stretched until the outer diameter was reduced to 430 to 440 μm. The diameters of the suture tip and of the stretched microtube were precisely sized and verified using a scale-fitted microscope (GZ6, Leica). The prepared suture was introduced into the stretched tubing so that 1.5 to 2 mm of its tip extended beyond the microtube. At the other side of the tube the suture was bent backward and the tube was connected to a supply tube (inner diameter 0.86 mm, outer diameter 1.52 mm; Portex) with an adapter tube in between (inner diameter 0.58 mm, outer diameter 0.96 mm; Portex), and all junctions were fixed with epoxide glue. Finally, the outside of the entire catheter device was coated with poly-L-lysine as previously described by Belayev and coworkers. A schematic drawing and a photograph of the catheter device are shown in Fig. 1.

Surgical Procedure

Male Sprague–Dawley rats weighing 270 to 330 g were anesthetized with 2% isoflurane in an 80%/20% air/O2 mixture using a face mask. The body temperature was maintained at 37°C using a heating pad. All animals received a subcutaneous injection of Atropin (0.01 mg/100 g body weight; Fresenius) and buprenorphine (3 μg/100 g body weight; Temgesic, Essex Pharma) to reduce mucus production and postoperative pain, respectively. A bur hole was drilled 2 mm posterior and 5 mm lateral to the bregma, leaving the dura mater intact. A small LDF probe (Moor Instruments) was placed on the dura and fixed to the skull with cyanoacrylate and dental cement. The LDF signal was continuously recorded and stored on hard disk using a purpose-designed multimodal monitoring system based on LabView software (National Instruments).

For selective MCA catheterization, the right CCA was exposed through a midline incision and carefully dissected from the surrounding tissue using microsurgery. The external carotid artery, lingual artery, and maxillary artery were ligated and cut. The ICA was exposed, and the origin of the pterygopalatine artery was visualized. The previously described catheter device was inserted via a small incision into the CCA and advanced into the ICA with special care not to enter the pterygopalatine artery (for a schematic representation, see Fig. 1). A sharp decrease in the LDF signal was interpreted to indicate successful MCA occlusion and catheterization. The catheter was fixed by a silk suture, and the neck incision was closed.

Selectivity of Intraarterial MCA Infusion

In three animals each, 50 or 500 μl saline solution containing 1% Evans blue (Sigma) was administered with a syringe pump at a flow rate of 100 μl/minute into the catheter device. Thereafter, animals were killed by exsanguination. The rats’ brains were immediately removed, and the skull and brain base were carefully inspected for the occurrence of subarachnoid hemorrhage as well as exact positioning of the catheter and thread. Photographs of the brain surface were taken to document MCA staining by Evans blue before the brains were frozen in chilled isopentane. Coronal sections (20 μm thick) were cut at 2000-μm intervals using a cryomicrotome (HM500, Microm) and maintained at −20°C. Photographs of the brain sections were taken using a digital camera, and extravasation of Evans blue was studied under a microscope (Axioplan 2, Zeiss) equipped with an appropriate filter set (excitation 546 nm, emission 590 nm) and a camera (AxioCam, Zeiss) for recording.

In three animals a short infusion of autologous blood adjusted to a hematocrit of 20% by dilution with saline was given via the catheter. In each animal three different flow rates were used: 100, 200, and 400 μl/minute under continuous control of the LDF signal. A recovery period was allowed after each infusion, during which the LDF signal returned to baseline. In another set of three animals the brain tissue PO2 was measured in the right MCA territory using a Licox probe (Integra NeuroScience). A bar hole was drilled 3 mm lateral and 0.5 mm anterior to the bregma, and after incision of the dura mater the Licox probe was implanted to a depth of 12 mm at a 45° angle directed posteriorly. The Licox probe was fixed to the skull with dental cement and connected to the monitoring system. The animals were then turned supine, and the catheter device was introduced as described earlier. Thereafter, Ringer solution or an artificial O2 carrier, the perfluorocarbon-bound blood substitute Oxycyte (Synthetic Blood International), was infused at different concentrations and flow rates. Both solutions were saturated with O2 prior to infusion. Flow was increased stepwise until the tissue O2 concentration reached a plateau. Before change to a different solution a 10-minute recovery period was established.

Induction of Focal Ischemia

For investigation of development of ischemia, five to seven animals per group were used (Table 1). Focal ischemia was induced by permanently inserting the catheter device to block the blood flow through the MCA. For comparison, a silicone-coated suture (mono- file 4-0 polyamide filament [Ethicon]; tip diameter 420–460 μm) was inserted as described earlier. The LDF decline was measured and recorded for 80 seconds after MCA occlusion. Thereafter, the LDF probe was disconnected, and the animals were allowed to recover from anesthesia and were transferred to their cages. Eight or 24 hours after MCA occlusion the rats again underwent induction of
TABLE 1
Total, subcortical, and cortical infarct volumes and the extent of swelling 8 and 24 hours after MCA occlusion with a conventional silicone-coated thread or the newly developed catheter device

<table>
<thead>
<tr>
<th>Occlusion (no. of rats)</th>
<th>Thread</th>
<th>Catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>8 hrs (7)</td>
<td>24 hrs (6)</td>
</tr>
<tr>
<td>total infarct volume (mm³)</td>
<td>348 ± 37</td>
<td>413 ± 38†</td>
</tr>
<tr>
<td>cortical infarct volume (mm³)</td>
<td>217 ± 34</td>
<td>225 ± 42</td>
</tr>
<tr>
<td>subcortical infarct volume (mm³)</td>
<td>132 ± 23</td>
<td>188 ± 18†</td>
</tr>
<tr>
<td>swelling (%)</td>
<td>16.9 ± 5</td>
<td>15.5 ± 10</td>
</tr>
<tr>
<td>swelling-corrected infarct volume (mm³)</td>
<td>289 ± 36</td>
<td>358 ± 33†</td>
</tr>
</tbody>
</table>

* Values are means ± SDs.
† p < 0.05 compared with 8 hours after occlusion with the respective method.

anesthesia and were killed by exsanguination. Their brains were immediately removed, and the base of the brain as well as the skull were carefully inspected. Animals appearing to have sustained a subarachnoid hemorrhage were excluded from further study. The brains were frozen in prechilled isopentane and stored at −80°C. Coronal cryosections (20 μm thick) were obtained at 500-μm intervals, air dried, and stained using the silver nitrate staining protocol described by Vogel and coworkers. The stained sections were scanned, and the infarct volumes were measured and analyzed using freeware image analysis software (Scion Image, Scion Corp.). In addition, the extent of swelling was calculated using the following equation described by Kaplan et al.; % swelling = 100 × (volume right hemisphere − volume left hemisphere) / volume left hemisphere.

Low-Dose MK-801 Application During Permanent Focal Ischemia: Selective Compared With Intravenous Injection

After establishing MCA occlusion by catheter placement in five animals per group, a 0.1-ml bolus of MK-801 (0.3 mg/kg body weight; Sigma) dissolved in saline was administered via the catheter device. A second bolus of 0.1 ml saline was given via the tail vein. In control animals the same dose of MK-801 was given via the tail vein while a 0.1-ml bolus of saline was slowly injected through the catheter device. Thereafter, animals were allowed to recover from anesthesia and were transferred to their cages. Twenty-four hours after MCA occlusion the animals again underwent induction of anesthesia; the aorta was cannulated to measure the mean arterial blood pressure and to withdraw an arterial blood sample for blood gas analysis. Thereafter, animals were killed, the brains removed, and the necrotic volume and extent of swelling were determined as mentioned earlier.

Statistical Analysis

Data are given as means ± SDs. For statistical analysis one-way analysis of variance and subsequent Fisher least significant difference testing were performed for comparison of ischemic volumes 8 and 24 hours after MCA occlusion with the catheter device or thread. For all other comparisons the Student t-test was used. Probability values less than 0.05 were considered statistically significant.

Results

Selective Intraarterial MCA Infusion

Insertion of the catheter via the ICA and careful advancement until correct placement was achieved resulted in an abrupt decrease in the LDF measurement to 23.9 ± 5.7% of baseline values. Selective perfusion of the MCA territory through the catheter device was shown by injection of 1% Evans blue solution. Autologous or synthetic O₂ carriers were used to document changes in physiological parameters such as cerebral blood flow and brain tissue PO₂.

A small bolus (50 μl) of 1% Evans blue selectively stained the MCA trunk and its main branches, whereas no staining was found in the arteries of the circle of Willis or in other pial vessels (Fig. 2). A short-term infusion of 500 μl dye solution (flow rate 100 μl/minute) showed substantial opening of the BBB and diffuse staining of the tissue in the cortical and subcortical MCA territory (Fig. 2).

Infusion of an autologous blood solution (hematocrit 20%) with increasing flow rates of 100, 200, and 400 μl/minute resulted in a flow-related increase in the LDF signal to 43.2 ± 10.2, 63 ± 13, and 101 ± 4.0%, respectively, of the baseline value recorded before positioning of the catheter device (Fig. 3). Infusion pauses were followed by an immediate return of the LDF signal to the level observed after catheter placement (Fig. 3). The brain tissue PO₂, which was 23.0 ± 9.9 mm Hg under baseline conditions, decreased to 5.5 ± 2.3 mm Hg after catheter placement. Infusion of oxygenated Ringer solution resulted in a small
flow-related increase in brain tissue PO$_2$ measured in the MCA territory (Fig. 4). A more pronounced increase was observed during infusion of oxygenated Oxycyte, and this increase was related to the flow rate and the concentration of the O$_2$ carrier (Fig. 4).

Induction of Focal Ischemia

Given that positioning of the catheter device results in MCA occlusion, we compared the development of ischemia with that induced by the conventional thread occlusion technique. The decrease in the measured LDF signal was comparable: we achieved 23.7 ± 9.2% of the preocclusion value with the catheter device and 21.5 ± 8.2% with the thread. The infarct volumes measured after 8 and 24 hours of MCA occlusion are listed in Table 1. We did not observe any differences in either the infarct volume or the extent of brain swelling. Furthermore, the increase in the infarct volume between 8 and 24 hours did not differ between animals that underwent occlusion with the catheter device and those with the thread.

Selective Intraarterial Low-Dose MK-801 Application During Permanent Focal Ischemia

The glutamate receptor antagonist MK-801 was used to test the efficacy of selective drug application to the endangered tissue. Twenty-four hours after MCA occlusion and drug infusion, monitoring of the blood pressure and acid base status in the arterial blood yielded the following values: 94.4 ± 8.4 mm Hg (mean arterial blood pressure), 7.41 ± 0.05 (pH), 47.1 ± 3.5 mm Hg (pCO$_2$), 88.2 ± 10.3 mm Hg (pO$_2$), and 43.2 ± 1.9% (hematocrit) without any difference between the treatment groups. However, the ischemic brain damage was significantly less in animals treated with selective infusion of MK-801 compared with systemic application. After 24 hours of MCA occlusion the total infarct volume was 508 ± 26 mm$^3$ after systemic and 339 ± 53 mm$^3$ after selective infusion of MK-801 (p < 0.001; Fig. 5). Similarly, the cortical and subcortical infarct volumes differed markedly (after systemic and selective application, the cortical infarct volumes were 313 ± 25 and 205 ± 27 mm$^3$, respectively [p < 0.001], and the subcortical infarct volumes were 195 ± 29 and 134 ± 50 mm$^3$, respectively [p < 0.05]). There was no difference in the extent of swelling (systemic application: 30.1 ± 8.7%, selective application: 23.5 ± 6.1%; p = 0.24). To eliminate the effect of brain edema, tissue necrosis was corrected for the degree of swelling in each animal. This analysis also confirmed the finding of significantly smaller total infarct volumes after selective application of MK-801 (269 ± 32 mm$^3$) compared with systemic drug application (354 ± 36 mm$^3$, p < 0.01).

Discussion

In the present study we describe a new model of super-
selective catheterization and highly controllable MCA perfusion in the rat. Using this model we blocked blood flow through the MCA by inserting a catheter into the CCA and advancing it into the ICA. This caused focal ischemia in the MCA, revealing the following findings: an early opening of the BBB which permits enhanced drug uptake as shown by Evans blue extravasation, alterations of physiological values such as cerebral blood flow and tissue oxygenation by selective infusion of autologous blood or a perfluorocarbon O2 carrier, development of tissue necrosis comparable to a conventional thread occlusion technique, and enhanced tissue protection during permanent focal ischemia by selective drug application when compared with systemic application.

The catheter device described here is a combination of the well-established intraluminal thread occlusion technique of the MCA and a custom-built microcatheter. The catheter device is advanced to block blood flow from the ICA and PCoA into the MCA by the tubing while flow from the ACA is discontinued by the tip of the filament that extends approximately 2 mm out of the catheter. Both the diameters of the suture tip and of the microtube were precisely controlled by a scale-fitted microscope and coated with poly-L-lysine to increase adhesion to the vessel wall as described previously.3

Selectivity of MCA Infusion

In previous experimental studies with selective drug application to the cerebrovascular system, solutions were infused into the intracranial part of the ICA,5,6,15 However, this approach does not allow the amount of solution entering the MCA to be estimated or controlled. Accordingly, supraphysiological flow rates had to be used for infusion. In contrast, the novel approach described in the present study allows selective infusion while totally blocking blood flow to the MCA. Thus, infusion of the test solution can reach the MCA territory exclusively. Selectivity of infusion was tested with injection of different volumes of Evans blue. A very small bolus (50 µl) adequately stained the MCA trunk and its main branches exclusively, indicating that the entrance into the ICA, the PCoA, and the ACA were tightly blocked by the catheter and the filament. In addition, a short-term infusion of Evans blue resulted in complete staining of the entire MCA territory with a sharp decrease in staining intensity at the border zone. The diffuse staining of the tissue suggests opening of the BBB very early after positioning of the catheter device, probably due to induction of hypoxia or ischemia. It is unlikely that the increased permeability was due to the 100-µl/minute flow rate because this is considerably lower than that expected under physiological conditions, assuming the following: a hemispheric volume of 600 µl (present results), an average blood flow of 120 µl/min/g tissue,24 and 50% of the hemisphere supplied by the MCA (calculated from the present results on ischemic volume). In previous studies Ding and coworkers5,6 perfused the MCA territory via a catheter positioned in the intracranial portion of the ICA in rats after a 120-minute MCA occlusion period. They infused a total of 7 ml saline at a flow rate of 2 ml/minute and assumed that half of the administered saline actually entered the MCA.5 This flow rate appears to be well above the physiological value and might, therefore, considerably increase edema formation. Unfortunately, these authors did not measure this parameter under their actual experimental conditions.

In addition to the dye we also infused autologous blood and a perfluorocarbon emulsion, Oxycyte, through the catheter device. Both cerebral blood flow measured by an LDF as well as the tissue oxygenation measured by a Licox probe increased in a flow rate–dependent manner. Thus, these observations agree with the results obtained by the dye and blood infusion experiments. The results also show that physiological parameters can be markedly affected even when small volumes are administered. Taken together, these experiments show that the present approach allows a truly selective infusion into the MCA of rats under well-controlled conditions.

Induction and Treatment of Focal Ischemia by Selective Drug Administration

In contrast to previously described rat models of selective intraarterial drug application to the cerebrovascular system,1,4,5,11,21,23,28,29 our new catheter device is accompanied by blockade of blood flow to the MCA and results in induction of focal brain ischemia when left in position for an extended period of time. We measured the decrease in the LDF signal and the development of ischemic brain damage and edema after 8 and 24 hours of occlusion. We then compared our findings with those of Koizumi and coworkers10 who first described the well-established thread occlusion technique. There were no differences with respect to any

![Bar graph showing the effect of low-dose MK-801 on infarct size in a permanent 24-hour MCA occlusion model. After establishing ischemia, superselective or intravenous infusion of MK-801 (0.3 mg/kg body weight) was performed. After 24 hours of permanent MCA occlusion the total infarct volume was significantly smaller in animals that received MK-801 selectively through the catheter device. The reduction in infarct volume occurred in the cortical and subcortical MCA territory to a comparable degree. Error bars denote the SDs. *p < 0.05, **p < 0.001 compared with systemic infusion.](image-url)
Selective MCA catheterization in rats

of these parameters, indicating that the blockade of blood flow was as complete as that achieved with a permanently applied thread.\textsuperscript{16,27}

The possibility of selectively perfusing the MCA while simultaneously blocking blood flow through the artery prompted us to test the effects of MK-801 infusion into the ischemic territory, a region normally insufficiently reached after systemic drug application. An N-methyl-D-aspartate-subtype glutamate-receptor antagonist, MK-801, has proven to be neuroprotective in several models of experimental stroke if given before or early after induction of ischemia.\textsuperscript{8,15,19} However, because of pronounced side effects of therapeutic doses the substance failed to earn clinical approval.\textsuperscript{16} In the present study we tested whether a fairly small dose (0.3 mg/kg body weight) of MK-801 delivered in 100 \(\mu\)l saline exhibits neuroprotective properties when specifically delivered to the ischemic territory. For comparison, a subset of animals received the same dose by intravenous injection along with infusion of 100 \(\mu\)l saline into the MCA as a solvent control. Selectively applying MK-801 significantly reduced the subcortical and cortical infarct volumes after 24 hours of permanent occlusion of the MCA while an intravenous injection of the same dose did not affect ischemic brain damage. This result supports our concept of a marked neuroprotective effect of small volume drug delivery to the ischemic territory even in the absence of reperfusion. Although we have not yet used our new catheter device in a model of transient MCA occlusion, there might well be additive effects of different treatment modalities when applied locally. Such investigations are of considerable clinical relevance, especially with respect to the use of endovascular approaches for clot lysis. During these interventions selective application of neuroprotective drugs into the ischemic territory would be possible in combination with local administration of plasminogen activators.

Conclusions

We have developed a new model of superselective catheterization of cerebral arteries that allows selective and well-controlled perfusion of the MCA. This approach is accompanied by an almost immediate opening of the BBB—affect that can be used to increase drug uptake and accumulation in the brain tissue. Evidence in favor of this is shown by the enhanced efficacy of a selectively applied glutamate receptor antagonist to the ischemic tissue. With reduced occlusion time (that is, without induction of ischemia by early catheter removal and early reperfusion), the catheter device might also be used to test new treatment modalities such as enhanced drug, gene-transfer, or stem-cell delivery in various other conditions affecting the brain such as brain tumors, vasospasm, or neurodegenerative disease.

References


J. Neurosurg. / Volume 106 / May, 2007 877


Manuscript received November 17, 2005. Accepted September 27, 2006.
Address reprint requests to: Dr. Johannes Woitzik, M.D., Department of Neurosurgery, University Hospital Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany. email: johannes.woitzik@nch.ma.uni-heidelberg.de.