

The macrosphere model

Evaluation of a new stroke model for permanent middle cerebral artery occlusion in rats

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Abstract

Background and purpose: The suture middle cerebral artery occlusion (MCAO) model is widely used for the simulation of focal cerebral ischemia in rats. This technique causes hypothalamic injury resulting in hyperthermia, which can worsen outcome and obscure neuroprotective effects. Herein, we introduce a new MCAO model that avoids these disadvantages. **Methods:** Permanent MCAO was performed by intraarterial embolization using six TiO₂ microspheres (0.3–0.4 mm in diameter) or by the suture occlusion technique. Body temperature was monitored, functional and histologic outcome was assessed after 24 h. Additional 16 rats were subjected to macrosphere or suture MCAO. Lesion progression was evaluated using magnetic resonance imaging (MRI). **Results:** The animals subjected to suture MCAO developed hyperthermia (> 39 °C), while the temperature remained normal in the macrosphere MCAO group. Infarct size, functional outcome and model failure rate were not significantly different between the groups. Lesion size on MRI increased within the first 90 min and remained unchanged thereafter in both groups. **Conclusions:** The macrosphere MCAO model provides reproducible focal cerebral ischemia, similar to the established suture technique, but avoids hypothalamic damage and hyperthermia. This model, therefore, may be more appropriate for the preclinical evaluation of neuroprotective therapies and can also be used for stroke studies under difficult conditions, e.g., in awake animals or inside the MRI scanner.

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Investigating the pathophysiology of ischemic stroke requires animal models for focal cerebral ischemia that are relevant to human stroke. Over the past decade, the intraluminal suture middle cerebral artery occlusion (MCAO) model, introduced by Koizumi et al. (1986), has been the most widely used technique. In this model, a monofilament is advanced into the internal carotid

artery (ICA) until its tip is lodged in the anterior cerebral artery (ACA) and thus blocks blood flow to the middle cerebral artery (MCA). Previous studies reported that this model induces hyperthermia (> 39 °C) early after MCAO. This pathological increase in body temperature is associated with hypothalamic injury, caused by an obstruction of the hypothalamic artery (HTA) as it originates from the distal ICA (Li et al., 1999; He et al., 1999; Reglodi et al., 2000; Zhao et al., 1994; Kuluz et al., 1993; Kiyota et al., 1993; Warner et al., 1993). It is widely accepted from experimental and clinical studies that hyperthermia increases infarct

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volume and worsens clinical outcome (Reglodi et al., 2000; Morikawa et al., 1992; Kim et al., 1996; Hajat et al., 2000; Chen et al., 1991; Reith et al., 1996; Dietrich et al., 1990; Azzimondi et al., 1995; Yanamoto et al., 2001) and, therefore, might influence the effects of neuroprotective treatments in animal studies (Memezawa et al., 1995).

The aim of this study was to develop and evaluate a new intravascular model for permanent MCAO in rats where hypothalamic injury and subsequent hyperthermia can be avoided. We intended to obstruct the MCA mainstem or the ICA distal to the origin of the HTA by intraarterial embolization using six ceramic microspheres 0.3–0.4 mm in diameter. We compared this new model with the established suture occlusion model with respect to infarct size and localization, body temperature, and clinical outcome and described the natural course of lesion development over time on diffusion-weighted magnetic resonance imaging (DWI) and perfusion-weighted magnetic resonance imaging (PWI) in both models.

1. Materials and methods

1.1. Animal preparation

All procedures used in this study are in accordance with our institutional guidelines. Male Sprague–Dawley rats weighing 290–350 g were caged in groups of two and acclimatized with a laboratory diet and tap water ad libitum under a fixed light–dark cycle for 2 weeks. The animals were anesthetized with an intraperitoneal injection of 250 mg/kg chloral hydrate. Anesthesia was maintained with 1% isoflurane delivered in air at 1.0 l/min. PE-50 polyethylene tubing was inserted into the left femoral artery for monitoring of blood pressure and for obtaining blood samples to measure pH, $p\text{CO}_2$, $p\text{O}_2$ and glucose. In animals subjected to magnetic resonance imaging (MRI) studies, additional PE-50 tubing was inserted into the left femoral vein for injecting gadopentetate dimeglumine (Magnevist, Berlex Laboratories, NJ) to perform cerebral perfusion analysis via bolus-tracking MRI. During the surgical procedure, body temperature was continuously monitored with a rectal probe and maintained at 36.5–37.0 °C with a thermostatically controlled heating lamp.

1.2. Induction of focal brain ischemia

The right common carotid artery (CCA), ICA and external carotid artery (ECA) were exposed through a midline incision of the neck. The ECA was isolated, and the superior thyroid and the occipital arteries were ligated with a 5–0 suture and transected. The distal portion of the ECA was ligated with a 3–0 suture and

transected to create an ECA stump with a length of approximately 5 mm. The pterygopalatine branch of the ICA was also ligated with a 5–0 suture.

1.2.1. Macrosphere model

Nylon tubing (ID: 0.5 mm; OD: 0.63 mm), filled with heparinized saline and six TiO_2 microspheres of 0.3–0.4 mm in diameter (BRACE GmbH, Alzenau, Germany) or with heparinized saline alone for sham procedure, was inserted into the ECA stump through an arteriotomy. During this procedure, the CCA and ICA were temporarily ligated with two 3–0 sutures for approximately 2 min to avoid hemorrhage. The tip of the tubing was placed in the carotid bifurcation without affecting the blood flow from the CCA to the ICA and fixed with a 5–0 suture. After the CCA and ICA ligations were released, the position of the tip of the tubing was corrected, if necessary, to avoid a restriction of the blood flow in the CCA/ICA. Then, the microspheres were advanced separately into the ICA by a slow injection of approximately 0.05 ml heparinized saline each, until they were moved passively into the cerebral circulation by the blood flow. Following the macrosphere injection, the CCA was again temporarily ligated and the ICA was carefully flushed with 0.5 ml of heparinized saline under simultaneous inspection of the carotid artery. Excessive dilatation of the ICA, indicating high flushing pressure, was avoided. The ICA and CCA were then temporarily ligated for approximately 20 s in order to remove the tubing and occlude the ECA stump with a prepared 5–0 ligation.

1.2.2. Suture model

A 4–0 silicone-coated nylon suture with a thermally rounded tip was introduced through an arteriotomy of the ECA stump as described previously (Li et al., 1999). The CCA and ICA were ligated temporarily for this procedure to prevent hemorrhage. The occluder was advanced into the ICA 16–18 mm beyond the carotid bifurcation until a mild elastic resistance indicated that the tip was properly lodged in the ACA and thus blocked blood flow to the MCA. After the occluder was fixed in place with a 5–0 suture, the ligations of the CCA and ICA were removed.

1.3. Experimental protocol

1.3.1. Part I

To evaluate the time course of body temperature, clinical outcome, 24 h mortality, infarct size and localization, and the technical failure rate in both models, we assigned 20 animals randomly to permanent MCAO using the macrosphere ($n = 10$) or the suture technique ($n = 10$).

Mean arterial blood pressure, pH, $p\text{CO}_2$, $p\text{O}_2$, body temperature and blood glucose were measured before

and 15 min after MCAO. The tubing was then removed from the femoral artery, the wounds were closed and the rats were allowed to recover from anesthesia. The body temperature was measured 1, 2, 3, 4, 5, 6 and 24 h after MCAO.

Neurological evaluation was performed 6 and 24 h after the induction of ischemia and scored on a 6-point scale, as described previously (Schabitz et al., 1999) which was modified from a scale proposed by Zea Longa et al. (1989): 0 = no neurological deficit, 1 = failure to extend left forepaw fully, 2 = circling to the left, 3 = falling to the left, 4 = no spontaneous walking with depressed level of consciousness and 5 = death.

Twenty four hours after induction of ischemia, the animals were deeply anaesthetized with an intraperitoneal injection of 1000 mg/kg chloral hydrate. The heart was punctured with a 20 G needle to derive a blood sample for blood glucose measurement. After decapitation, the brains were quickly removed and the localization of the microspheres in the basal cerebral arteries was examined by visual inspection using a magnifying glass. The brains were sectioned coronally into six slices, each 2 mm in thickness, beginning from the frontal pole of the cortex. The slices were incubated in a 2% solution of 2,3,4-triphenyltetrazolium chloride (TTC) at 37 °C, fixed by immersion in 10% buffered formalin solution and scanned with a coupled CCD camera (ECD-1000HR computer camera, Electrim Corp.). The unstained areas of the fixed brain slices were defined as infarcted (Benderson et al., 1986; Li et al., 1997). Using image analysis software (Bio Scan Optimas), the areas of the infarcted regions and of both hemispheres were calculated for each coronal slice. The percent hemispheric lesion volume (%HLV) was calculated to compensate for the space-occupying effect of brain edema, using the equation

$$\%HLV = \frac{LV}{HV_i},$$

where LV is the direct lesion volume and HV_i the ipsilateral hemispheric volume, both calculated by multiplying the area by the slice thickness of the central four slices and summing the volumes from all slices (Lin et al., 1993). Hypothalamic damage was determined from the TTC-stained brain slices as described previously (Li et al., 1999). Determination of infarct size and hypothalamic injury was performed by an experienced investigator without the knowledge of group assignment.

In the macrosphere model, appropriate MCAO was considered to be present, if one or more microspheres directly blocked the MCA mainstem or if the distal ICA and the ACA were occluded, blocking blood flow into the MCA (Fig. 1A). Animals were excluded, if the microspheres were localized in the distal ICA beyond

1.5 mm in length from the MCA orifice, because that is most likely to block the HTA from the ICA, resulting in a hypothalamic infarction, based on our pilot studies (see Fig. 1A). In the suture model, appropriate MCAO was confirmed by postmortem verification of the occluder position (Fig. 1B). Animals were excluded from this study if the tip of the occluder was not lodged properly in the ACA or if an intracerebral or subarachnoidal hemorrhage was present.

Sham operation was performed in three animals in accordance with the macrosphere-MCAO protocol by injecting heparinized saline without microspheres into the carotid artery.

1.3.2. Part II: magnetic resonance imaging

Sixteen animals were randomized to the macrosphere MCAO group ($n = 8$) or the suture MCAO group ($n = 8$). Mean arterial blood pressure, pH, pCO_2 , pO_2 and body temperature were measured before and 60 min after MCAO.

The animals were fixed in a body holder with ear-bar and tooth-bar restraints and were placed in an MRI spectrometer. Anesthesia was maintained with 1.0% isoflurane delivered in air at 1.0 l/min. Temperature was monitored using a rectal probe and maintained at 36.5–37 °C by a thermostatically regulated airflow system during the entire imaging protocol.

The MRI spectrometer (GE CSI-II 2.0 T/45 cm; General Electric NMR Instruments, Fremont, CA) operating at 85.56 MHz for the 1H and equipped with ± 20 G/cm self-shielding gradients. Multislice, diffusion-weighted spin-echo echo-planar imaging (EPI) was used to map the apparent diffusion coefficient (ADC) of brain tissue water. Eight contiguous, coronal slices with a thickness of 2 mm, were acquired with a field-of-view (FOV) of 25.6 mm \times 25.6 mm and a matrix size of 64 \times 64 (repetition time (TR) = 5 s, echo time (TE) = 74 ms, EPI data acquisition time = 65 ms, number of excitations (NEX) = 2, diffusion-sensitive gradient pulse width (δ) = 7 ms, and diffusion-sensitive gradient separation time (Δ) = 35 ms). The imaging offset in the coronal plane was adjusted such that the second slice started from the frontal pole of the brain. Half-sine-shaped diffusion-sensitive gradient pulses were applied along one of the three orthogonal gradient axes (x , y or z). For each slice, nine b -values, ranging from 18 to 1552 s/mm², were used to measure the ADC of water along each of the three diffusion-gradient directions. Using linear least-squares regression analysis, the natural logarithm of the signal intensity was fitted to the b -values where the slope of the fitted line is proportional to the ADC. The average ADC map was calculated by averaging the three orthogonal ADC values on a pixel-by-pixel basis (van Gelderen et al., 1994). The four central slices of the diffusion data (at 2–8 mm from the frontal pole) were used to calculate the lesion volume.

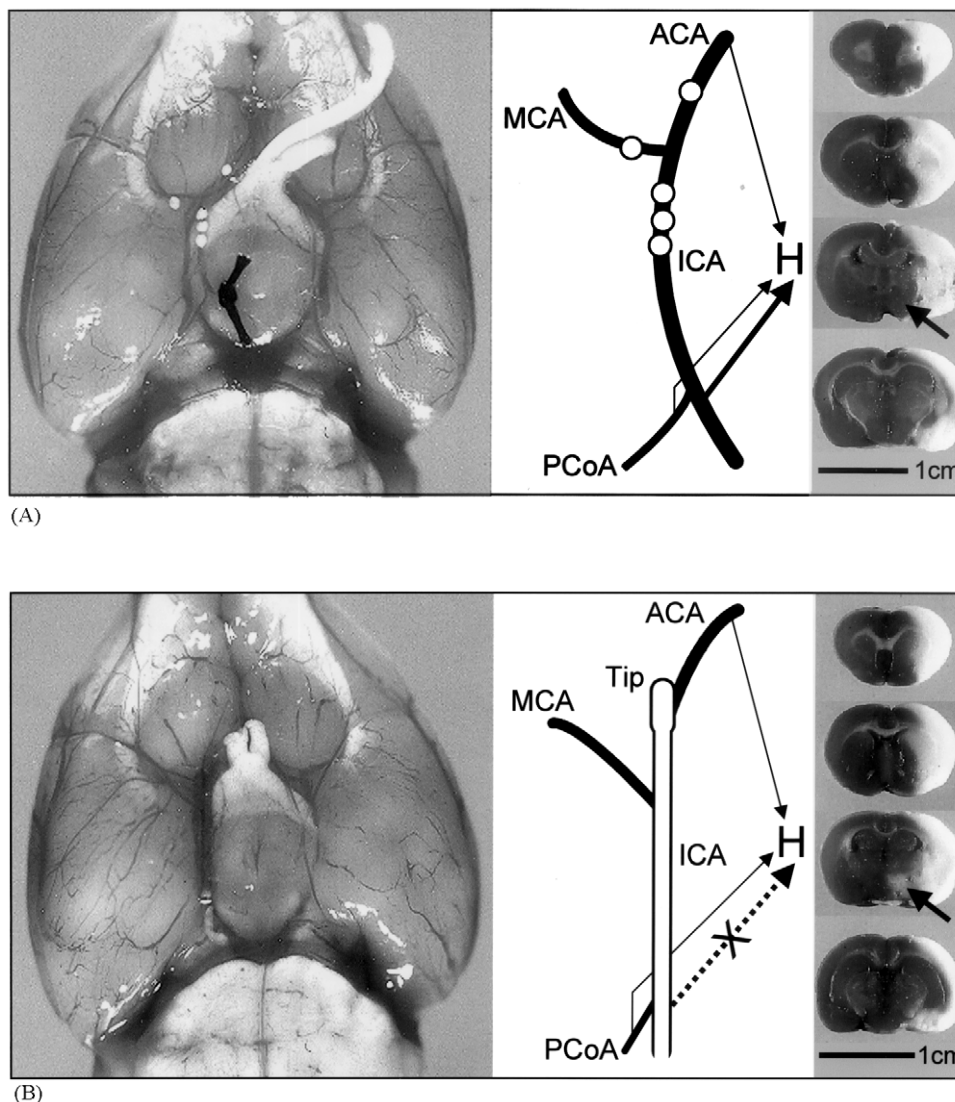


Fig. 1. (A) MacroSphere MCAO. Left: Photography of the basal cerebral arteries following macroSphere-induced MCAO. The macroSpheres are located in the MCA, ACA and the distal ICA within the first 1.5 mm from the MCA orifice. Animals were excluded, if the macroSpheres were localized in the distal ICA beyond 1.5 mm in length from the MCA orifice (*), to avoid an occlusion of the HTA. Middle: Diagram of the ipsilateral cerebral arteries. The blood supply to the hypothalamus (arrows) from the ICA and PCoA is not affected by the macroSpheres. The ACA and its branches to the hypothalamus were perfused from the contralateral hemisphere via the anterior communicating artery (crossflow). Right: TTC staining of the central four slices 24 h after MCAO reveals an infarction of the MCA territory. No ischemic lesion could be detected in the hypothalamic area (arrow). (B) Suture MCAO. Left: Photography of the basal cerebral arteries following suture-induced MCAO. The suture is located in the ICA and the proximal ACA and thus blocks the blood flow to the MCA. Middle: Diagram of the ipsilateral cerebral arteries. The blood supply to the hypothalamus originating from the ICA is blocked by the suture (\times). Hypothalamic branches from the ACA were perfused from the contralateral hemisphere via the anterior communicating artery (crossflow). The hypothalamic blood supply originating from the PCoA is not affected. Right: The TTC staining of the central four slices 24 h after MCAO demonstrates an infarction of the MCA territory. The arrow indicates the ischemic lesion of the hypothalamus. ICA = distal internal carotid artery; MCA = middle cerebral artery; ACA = anterior cerebral artery; PCoA = posterior communicating artery; HTA = hypothalamic artery; Tip = tip of the suture; H = hypothalamus.

T2-weighted EPI was used to perform dynamic contrast-enhanced PWI (Wendland et al., 1991). Four contiguous coronal slices with a 2 mm thickness, which corresponded to the central four diffusion slices, were acquired with an FOV of 25.6 mm \times 25.6 mm and a matrix size of 64 \times 64. A total of 40 spin-echo EPIs (TR = 900 ms, TE = 74 ms, EPI data acquisition time = 65 ms, NEX = 1) was obtained for each slice. A bolus

injection of 0.25 ml of gadopentetate dimeglumine was administered after the fifteenth image acquisition.

For each animal, DWI and PWI were acquired 30, 60, 90, 120, 180 and 240 min after MCAO. To define abnormal ADC values, each pixel in the affected hemisphere was compared with its corresponding pixel in the normal hemisphere and the percent difference in ADC (Δ ADC) was calculated. To differentiate normal from

pathologic tissue, a threshold of -29% Δ ADC was used as described previously (Takano et al., 1996). The %HLV was calculated from the central four slices, by dividing the abnormal volume by the volume of the affected hemisphere. Edema correction was not performed, since the space-occupying effect of brain edema in the very early stage of focal cerebral ischemia might be negligible.

Perfusion-weighted images were evaluated qualitatively by visual inspection. The size and localization of the perfusion deficit were determined by an experienced investigator without knowledge of the assignment of the animal group.

After the MRI experiments, tubing was removed from the femoral artery and vein, the wounds were closed and the rats were allowed to recover from anesthesia. Neurological evaluation and TTC staining was performed 24 h after induction of ischemia as described in Section 1.3.1.

1.4. Statistical analysis

Data are presented as mean \pm S.D. Physiological variables were compared with the use of a two-tailed *t*-test, non-parametric variables with a Mann–Whitney *U*-test. The DWI- and TTC-derived lesion volumes were compared using a linear regression analysis. Significance was set at $P < 0.05$.

2. Results

2.1. Part I

The physiological variables (Table 1) did not differ between the macrosphere- and suture-occluded animal groups at any time point. Furthermore, no statistical difference among the physiological variables could be observed before and after MCAO within each group ($P > 0.05$; *t*-test).

All animals were fully awake 30–45 min after MCAO and were able to maintain their body temperature above 36.5 °C without external heating. In the macrosphere group, body temperature remained within the physiological range (36.5 – 37.5 °C) at all time points after MCAO. Animals subjected to suture MCAO showed an elevated temperature, reaching a maximum of 39.2 °C (mean) 3 h after MCAO (Fig. 2). The difference in temperature between both groups was significant as early as 1 h after MCAO ($P < 0.05$), became highly significant after 2–6 h ($P < 0.0001$) and was still significant after 24 h ($P < 0.05$; *t*-test). The mean neurological score did not differ between the groups at any time point ($P > 0.05$; *U*-test). No abnormal behavior (e.g., seizures) could be observed. In each group,

one animal died between 6 and 24 h after MCAO (Table 2).

The appearance of the macrospheres in the basal cerebral arteries was clearly visible in all animals of the macrosphere MCAO group. Macrospheres could be detected in the MCA mainstem (9/10 animals), the ACA (7/10), the distal portion of the ICA (4/10) or in the posterior communicating artery (8/10; Fig. 1A). No macrospheres were identified in distal MCA branches or in the contralateral hemisphere.

Three animals in the macrosphere group were excluded and replaced. In two rats, all macrospheres were lodged in the ICA proximal to the MCA orifice, thus, without occluding the MCA or ACA. TTC staining of these two animals revealed small subcortical lesions in the distribution of the hypothalamic and the anterior choroidal arteries with lesion sizes of 18 and 38 mm³, respectively. In one rat, all macrospheres were lined up from the MCA orifice to the distal ICA beyond 1.5 mm in length, thus occluding both the MCA orifice and the HTA as well. TTC staining revealed a lesion of the MCA territory and the hypothalamus. These three animals developed hyperthermia similar to the animals in the suture MCAO group. In all animals subjected to macrosphere MCAO that fulfilled the inclusion criteria, TTC staining revealed infarctions in the MCA territory, always involving both the cortex and the underlying caudoputamen, but sparing the hypothalamus (Fig. 1A).

In the suture group, two animals were excluded and replaced. In one rat, the depth of the suture insertion was not appropriate, resulting in a small, isolated hypothalamic infarction (lesion size < 20 mm³) without cortical involvement. The second animal was excluded because of a subarachnoid hemorrhage caused by a perforation of the ACA. All other animals in the suture group developed infarctions in the MCA territory, including the cortex, the caudoputamen and the distribution of the anterior choroidal and HTA.

The %HLV ($45.9 \pm 7.0\%$ in the suture group and $40.2 \pm 13.4\%$ in the macrosphere group) was not significantly different ($P > 0.05$; *t*-test).

In the three sham-operated animals, physiological variables and body temperature remained within the physiological range. No neurological deficits could be observed after 6 and 24 h and no pathological findings were found at postmortem examination or on the TTC staining.

2.2. Part II

All eight animals in the suture MCAO group and seven of eight animals in the macrosphere group survived 24 h. In one animal subjected to the macrosphere MCAO, spheres were lodged proximal to the MCA orifice. This animal was therefore replaced according to the exclusion criteria. The physiological

Table 1
Physiological variables in animals subjected to macrosphere or suture MCAO

	Baseline					15 min after MCAO					24 h		
	BW (g)	MABP (mmHg)	Gluc (mg%)	pO ₂ (mmHg)	pCO ₂ (mmHg)	pH	MABP (mmHg)	Gluc (mg%)	pO ₂ (mmHg)	pCO ₂ (mmHg)	pH	Gluc (mg%)	
Macrosphere MCAO	323 ± 22	103 ± 10	141 ± 37	92 ± 13	43 ± 3	7.40 ± 0.01	99 ± 13	125 ± 24	85 ± 3	39 ± 5	7.41 ± 0.03	158 ± 30	
Suture MCAO	321 ± 18	99 ± 10	168 ± 32	90 ± 10	44 ± 1	7.41 ± 0.02	95 ± 15	157 ± 37	83 ± 4	40 ± 27	7.43 ± 0.02	156 ± 36	

Values are expressed as mean ± S.D. BW, MABP and Gluc indicate bodyweight, mean arterial blood pressure and blood glucose, respectively.

variables (data not shown) did not differ between both groups at any time point or before and after MCAO in each group ($P > 0.05$; t -test).

2.3. Perfusion deficits on PWI

Hyperintense regions on PWI that corresponded with the absence of gadopentetate dimeglumine in the cerebral vasculature indicate hypoperfused tissue. PWI revealed hyperintense regions in the right caudoputamen and the overlying cortex in both models as early as 30 min after MCAO. In contrast to the macrosphere MCAO group, the area of hyperintensity in the suture MCAO group included the hypothalamic region. No apparent changes of the localization and size of the perfusion deficits were observed over time, indicating the absence of premature reperfusion.

2.4. Lesion progression on DWI

In both groups, DWI hyperintensity was observed in the distribution of the right MCA, mainly involving the lateral caudoputamen and the overlying cortex at all time points. An additional DWI lesion in the hypothalamic region could be identified in the suture MCAO group (Fig. 3B). In the macrosphere group, the mean %HLV increased from $31.6 \pm 14\%$ (30 min after MCAO) to $51.3 \pm 8\%$ (240 min). In the suture MCAO group, lesion volume increased from $29.8 \pm 10\%$ (30 min) to $46.8 \pm 14\%$ (240 min). Compared with the lesion size at 4 h, DWI lesions were significantly smaller at 30, 60 and 90 min ($P < 0.05$), but not at 120 and 180 min after ischemia in both groups ($P > 0.05$; t -test). No significant difference of %HLV could be observed between the groups at any time point (Fig. 3A).

2.5. Postmortem findings

Inspection of the basal cerebral arteries confirmed an appropriate localization of the sutures in all animals and of the macrospheres in all but one animal. In rats subjected to macrosphere MCAO, spheres could be identified in the MCA mainstem (all animals), in the ACA (7/8 animals), in the distal ICA (6/8) and in the posterior communicating artery (6/8). Twenty-four hours after ischemia, TTC staining of the corresponding brain slices revealed an infarct size similar to the DWI-derived lesion volumes at 4 h (macrosphere: 47.5%; suture: 49.4%). Both methods correlated significantly between the 4-h DWI %HLV and the postmortem lesion volume (macrosphere: $r = 0.88$, $P < 0.05$; suture: $r = 0.83$, $P < 0.05$; linear regression). Hypothalamic lesions were detected in all animals subjected to suture MCAO but only in one rat subjected to the macrosphere MCAO in whom the macrospheres were located in the distal ICA beyond 1.5 mm in length from the MCA orifice and

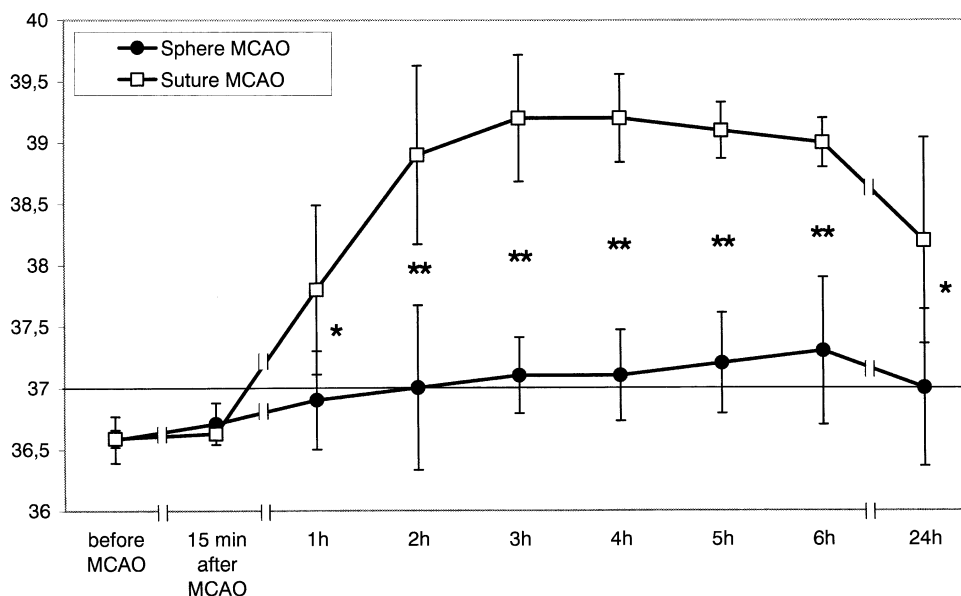


Fig. 2. Body temperature. Time course of mean body temperature (\pm S.D.). A significant difference in body temperature could be detected as early as 1 h after MCAO between both models. Asterisks indicate a significant difference between both groups (* $P < 0.05$; ** $P < 0.0001$).

thus blocked the HTA. This animal developed elevated body temperature after surgery. According to our exclusion criteria, this rat was therefore excluded and replaced.

3. Discussion

Clinical studies demonstrate highly inhomogeneous vascular findings in patients suffering from acute territorial stroke, ranging from early recanalization (i.e., “normal” vascular status upon admission) or delayed or drug-induced recanalization (i.e., within the first hours or days) to permanent vessel occlusion (del Zoppo et al., 1998; Furlan et al., 1999; Gerriets et al., 2000). The underlying pathophysiology of ischemia-induced brain damage therefore is likewise heterogeneous, depending on the presence (and time point) of additional reperfusion injury (Jean et al., 1998; Kuroda and Siesjo, 1997; Dietrich, 1994). Consequently, experimental stroke studies should be performed in both,

transient and permanent vessel occlusion, to simulate closely the heterogeneous conditions in patients, as recommended by the STAIR roundtable (Stroke Therapy Academic Industry Roundtable, 1999). The established suture MCAO model provides a highly reproducible induction of focal cerebral ischemia in rodents and allows reperfusion at defined time points. Permanent MCA occlusion with this model, however, causes hypothalamic damage and subsequently hyperthermia, with potential adverse effects on experimental results (Li et al., 1999; Reglodi et al., 2000; Zhao et al., 1994; Memezawa et al., 1995), since hypothalamic infarction is extremely rare in patients. Thus, we developed a new model for permanent MCA occlusion that avoids this major disadvantage of the permanent suture technique.

The intraarterial embolization of microspheres (0.3–0.4 mm in diameter) provides a reproducible occlusion of the MCA mainstem, resulting in focal ischemic lesions that are comparable with the suture model. However, this technique preserves the blood supply to

Table 2
Functional and histological outcome in animals subjected to macrosphere or suture MCAO

	Neurological score 6 h after MCAO	Neurological score 24 h after MCAO	Lesion volume (%HLV)	Hypothalamus infarction	24 h mortality	Model failure rate
Macrosphere	1.7 \pm 0.5	1.8 \pm 1.2	40.2 \pm 13.4	0/10	1/10	3/13 ^a
Range	1–2	1–5	21.1–59.0			
Suture	1.7 \pm 0.5	2.3 \pm 1.3	45.9 \pm 7.0	10/10	1/10	2/12 ^a
Range	1–2	1–5	36.7–54.1			

^a Three animals were excluded in the macrosphere group due to inappropriate localization of the microspheres, resulting in an additional hypothalamus infarction in one and no ischemic deficit in two animals. In the suture MCAO group, the occluder failed to block MCA blood flow in one animal and caused a perforation of the ACA with consecutive subarachnoidal hemorrhage in another rat. All excluded animals were replaced.

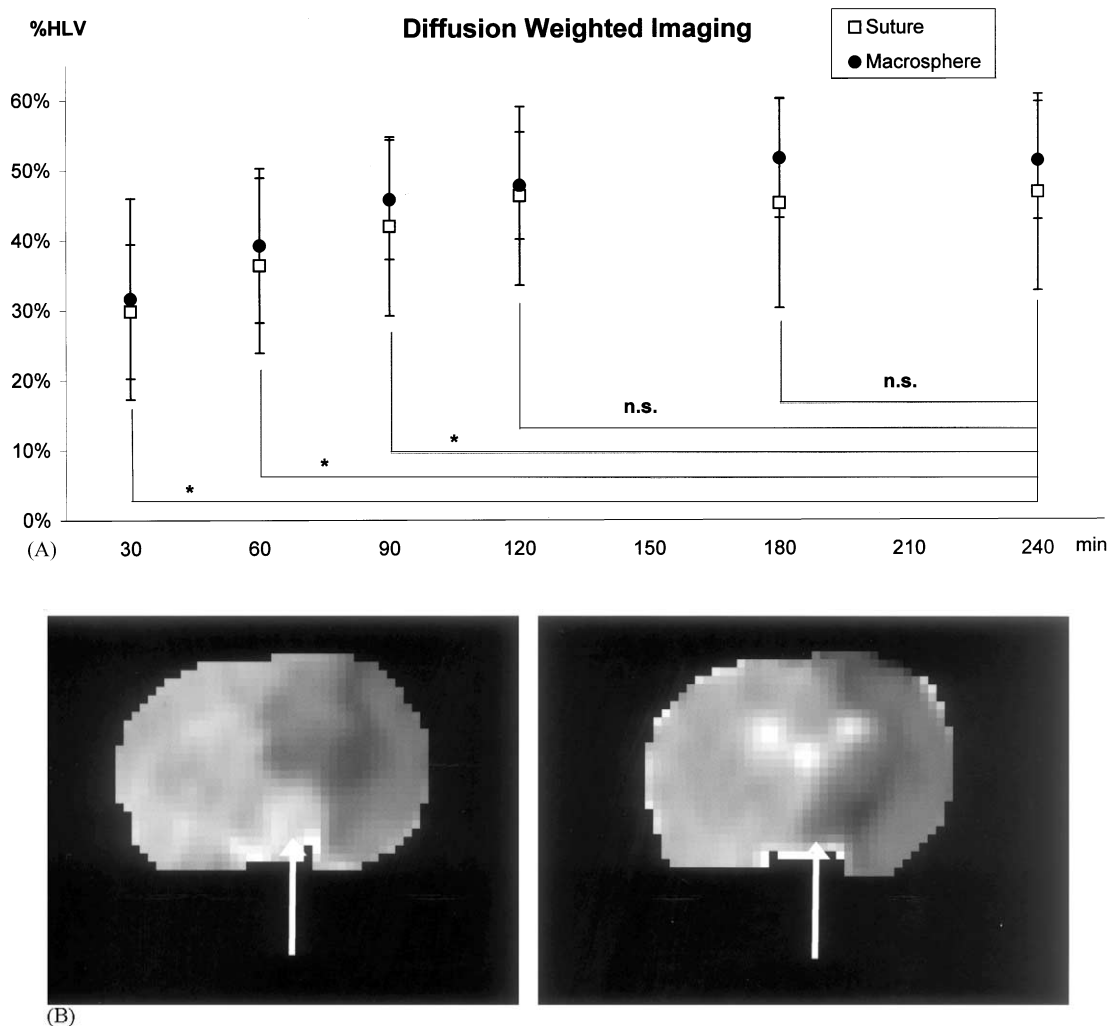


Fig. 3. (A) Lesion progression on DWI. Lesion size increased until 90 min after onset of ischemia in both groups and remained constant thereafter. %HLV indicates hemispheric lesion volume \pm S.D. The asterisks indicate a significant difference of %HLV at different time points compared with %HLV at 240 min ($P < 0.05$). n.s. indicates not significant ($P > 0.05$). (B) ADC maps. Representative ADC maps of two animals, subjected to macrosphere MCAO (left) and suture MCAO (right). The ischemic lesion involves the hypothalamic region only, if the suture technique was applied (arrows).

the hypothalamus and thus avoids hypothalamic infarctions and resulting hyperthermia.

In pilot studies (data not shown), we tested microspheres of different size and material. TiO_2 microspheres of 0.13–0.20 mm in diameter frequently moved into distal MCA branches. In some cases, this resulted in very small subcortical ischemic damage. However, in most cases no lesions could be detected on TTC stains, most likely due to the extensive collateralization among the major MCA branches in Sprague–Dawley rats (Rubino and Young, 1988; Niiro et al., 1996). Approximately, 70% of Al_2O_3 microspheres (0.25–0.3 mm) could be detected in the desired localization while the remaining microspheres also moved into smaller MCA branches. Using six Al_2O_3 microspheres, two-thirds of

the animals developed a large MCA stroke. A smaller number of these microspheres did not result in reproducible ischemic lesions, while larger numbers typically filled a long segment (more than 1.5 mm) of the distal ICA and therefore occluded the HTA, similar to what is observed in the suture model. The best results could, therefore, be achieved with six TiO_2 microspheres of 0.3–0.4 mm in diameter. Larger spheres (e.g., 0.4–0.6 mm) could not be embolized into the basal cerebral arteries.

We deposited the microspheres into the carotid bifurcation, using an approach through the ECA. The microspheres were carried passively into the cerebral circulation by the blood flow in the ICA. This technique, therefore, simulates closely artery–artery or cardioem-

bolic embolization, which are regarded to be the most frequent pathophysiological mechanisms in human territorial stroke.

We compared our new model with the established suture MCAO technique, that was first described by Koizumi et al. (1986), and has been modified by Li et al. (1999) and various other groups (Memezawa et al., 1992; Belayev et al., 1996; Garcia et al., 1995; Nagasawa and Kogure, 1989). This technique provides highly reproducible ischemic lesions and serves as the “gold standard” for the induction of focal cerebral ischemia. Technical failure rate, infarct volumes and clinical outcome are comparable in both techniques with a slightly higher standard deviation of infarct size in the macrosphere group. The latter finding is most likely a result of the random distribution of macrospheres in the basal cerebral arteries.

DWI and PWI revealed almost identical findings with both MCAO techniques. Hyperintense regions on PWI were detected on the first images (30 min after MCAO) in all rats and remained detectable on follow up. PWI, therefore, serves as an indicator for a sufficient induction of ischemia in an early stage of stroke experiments and can therefore be used as an exclusion criterion before randomization to a drug treatment.

The lesion size on DWI increased during the first 90 min and remained stable thereafter, which is in accordance with our previously published experience with the suture MCAO model (Reith et al., 1995). However, the temporal evolution of DWI lesions was essentially the same in the suture MCAO group and the macrosphere MCAO group, which reflects the identical underlying pathophysiology (occlusion of the MCA mainstem) in the two models.

These findings provide evidence that TiO₂ microspheres block the MCA blood flow immediately after embolization and that premature reperfusion, e.g., due to a dislocation of the microspheres, is unlikely to occur.

As expected, all animals assigned to the suture model group developed hypothalamic lesions and subsequent hyperthermia. In contrast, hypothalamic injury with a pathological increase of body temperature was observed in only one animal in each macrosphere MCAO group.

Several groups reported hyperthermia > 39 °C as a constant finding early after permanent MCA occlusion in rats using the intraluminal suture technique (Li et al., 1999; He et al., 1999; Reglodi et al., 2000; Zhao et al., 1994; Kuluz et al., 1993; Kiyota et al., 1993; Warner et al., 1993). Li et al. demonstrated that this phenomenon also occurs in transient MCAO, if the duration of ischemia exceeds 90 min. In this study, hyperthermia was always accompanied by an ischemic lesion of the hypothalamic region. Hyperthermia also occurred in rats with an isolated infarction of the hypothalamus (Li et al., 1999; He et al., 1999).

The hypothalamus is considered as the most important center of thermoregulation in mammals. Its blood supply is derived mainly from the HTA that originates from the distal portion of the ICA and from long, perforating branches of the ACA. The caudal parts of the arcuate nuclei of the hypothalamus also receive branches from the posterior communicating artery (Fig. 1). The ventromedial preoptic nucleus, that receives its blood supply from the distal ICA, plays a key role in hypothalamic thermoregulation (He et al., 1999; Scremin, 1970; Milton, 1978; Saper, 1998). Models for focal cerebral ischemia using the suture technique lead to an occlusion of the ICA from the bifurcation to the origin of the ACA and MCA and therefore block the HTA, while the blood supply to the hypothalamus derived from the ACA or the posterior communicating artery might persist to some extent due to a crossflow from the contralateral hemisphere (Fig. 1). Indeed, these anatomic features could explain the long resistance to ischemia (≤ 90 min) and the reversibility of hypothalamic lesions on DWI after 90 min of MCAO, as a result of the extensive collateral blood supply of the hypothalamus (Li et al., 1999). However, a prolonged occlusion of the HTA (e.g., > 90 min) results in an irreversible hypothalamic injury that is accompanied by an increase in body temperature (Li et al., 1999; Zhao et al., 1994; Kuluz et al., 1993; Kiyota et al., 1993; Warner et al., 1993; Schmidt-Elsaesser et al., 1998). Other stroke models, that do not affect the hypothalamic blood supply, such as the direct electrocoagulation of the MCA, seem not to induce hyperthermia (Yamashita et al., 1997).

Many previous studies indicate that hyperthermia increases infarct size and worsens clinical outcome (Reglodi et al., 2000; Kim et al., 1996; Chen et al., 1991; Reith et al., 1996; Dietrich et al., 1990; Azzimondi et al., 1995; Yanamoto et al., 2001). However, in Section 2.1 of our study we found only a non-significant trend towards a larger lesion size in (hyperthermic) animals subjected to suture MCAO, compared with the normothermic macrosphere group (45.9%HLV vs. 40.2%HLV). This difference was less pronounced in the MRI study, where body temperature in all animals was maintained at 37.0 °C for the first 4.5 h after MCAO (49.4%HLV vs. 47.5%HLV).

Furthermore, induced hyperthermia might influence the results of experimental stroke studies. In a study reported by Memezawa et al., the efficacy of NMDA antagonist MK-801 was evaluated as a neuroprotective drug. Using the suture technique, rats were subjected to MCAO for 2 h and body temperature was allowed to rise spontaneously to 39–39.5 °C or maintained at 37 °C by external cooling. Only in the latter experimental group was MK-801 found to be neuroprotective, highlighting the importance of preventing hyperthermia in neuroprotective drug studies (Memezawa et al., 1995).

Two animals in the suture MCAO group and three animals in the macrosphere group were excluded because of meeting our exclusion criteria. In the suture model, exclusion criteria that are based on the site of occlusion can be derived from an inspection of the position of the occluder tip during the removal of the brain. However, any mechanical manipulation of the suture during this procedure has to be avoided carefully. In the macrosphere model, vessel occlusion can be verified easily on postmortem evaluation. The localization of the microspheres in the basal cerebral arteries can serve as a simple and valid exclusion criterion. Furthermore, typical complications of the suture model, such as subarachnoidal hemorrhage or inadvertent reperfusion are unlikely to occur in the macrosphere model (Schmidt-Elsaesser et al., 1998).

Non-endovascular techniques for the induction of focal cerebral ischemia, e.g., the direct microsurgical occlusion of the MCA, do not impair the hypothalamic circulation (Yamashita et al., 1997). Nevertheless, these methods require a craniotomy and are, therefore, more invasive, can alter intracranial pressure, hemodynamics and local brain temperature and may cause parenchymal and vascular injuries. Furthermore, a craniotomy can lead to artifacts in *in vivo* imaging techniques (Robinson, 1979; Tamura et al., 1981; Hudgins and Garcia, 1970; Olessen, 1987). Endovascular models, other than the suture technique, consist in the intraarterial embolization of microspheres, usually calibrated between 0.03 and 0.08 mm. This “microsphere model” induces confluent focal ischemic lesions, but the underlying pathophysiological concept of occluding the cerebral microcirculation limits a simulation of human territorial stroke (Bralet et al., 1979; Demura et al., 1993; Miyake et al., 1993).

In summary, the macrosphere model is a reproducible technique for permanent MCAO that is comparable with the established suture model with respect to infarct development over time, final lesion size and localization and clinical outcome, but avoids hypothalamic infarctions with resulting postischemic hyperthermia. Thus, this model represents a closer simulation of the pathophysiology of human stroke and can be helpful in the preclinical evaluation of neuroprotective therapies. Furthermore, due to its relatively simple and practical methodology, this model might be useful for experimental stroke studies under difficult conditions, e.g., for MCAO in awake animals or inside the MRI scanner.

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