Transfemoral selective “intraluminal wiring” technique for transient middle cerebral artery occlusion in rats

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Received 13 December 2004; received in revised form 19 April 2005; accepted 9 May 2005

Abstract

While the intraluminal thread technique to induce middle cerebral artery occlusion is widely used in animal models of focal cerebral ischemia, it has several drawbacks. The present study describes a new technique involving transfemoral selective intraluminal wiring, and evaluates its technical feasibility, effectiveness, and safety. Twenty-four Wistar rats were used in this work: two for a vascular anatomy study and 22 subjected to middle cerebral artery occlusion for 1 h by our new transfemoral selective “intraluminal wiring” technique. After 24 h of reperfusion, the animals were evaluated neurologically, and then were sacrificed. Macroscopic, histological (2,3,5-triphenyltetrazolium chloride (TTC), hematoxylin–eosin and TUNEL), and biochemical (DNA fragmentation and caspase-3 activity) studies were performed to assess the extent of brain damage produced by focal ischemia. Technical success was obtained in all 22 animals. Signs of focal ischemia and reperfusion, such as necrosis and apoptosis, were detected in the middle cerebral artery territory. No subarachnoid hemorrhage was noticed in any animal. Transfemoral selective intraluminal wiring appears to be a reliable, safe, and minimally invasive technique to induce transient focal cerebral ischemia in rats.

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Keywords: Apoptosis; Intraluminal wiring; Middle cerebral artery; Necrosis; Transient focal ischemia; Wistar rats

1. Introduction

The development of a reliable animal model of cerebral ischemia is essential to studying the pathophysiology of stroke and to evaluating new therapies for this the commonest cause of permanent disability severely impairing patients’ quality of life. The intraluminal thread model of middle cerebral artery (MCA) occlusion in rats—first described by Koizumi et al. (1986), and subsequently modified by other workers (Longa et al., 1989; Belajev et al., 1990)—has been extensively used and studied. This technique has the advantages of being relatively non-invasive, eliminating the need for craniotomy with its associated operative trauma, and of the potency of the internal carotid artery after removal of the thread to permit reperfusion. However, the technique has several inherent drawbacks that have yet to be resolved. These include insufficient MCA occlusion, inadvertent premature reperfusion, and subarachnoid hemorrhage (SAH) (Longa et al., 1989; Luang et al., 1993; Kuge et al., 1995; Bederson et al., 1995; Schmid-Elsaesser et al., 1998). More recently, a new technique to induce MCA occlusion has been described using intra-arterial embolization with ceramic macroparticles (Gerriets et al., 2004). This proved to reliably establish cerebral ischemia without SAH, but led to permanent MCA occlusion that limits reperfusion. In the present study, we describe a new technique of transfemoral selective “intraluminal wiring” and its application in producing MCA occlusion in rats. Selective intraluminal wiring, modified from the selective catheterization technique in interventional radiology, is defined as selective endovascular placement of a
guidewire into a target vessel under fluoroscopic guidance, but without the use of a catheter or an iodinated contrast agent. The purpose of the present study was to evaluate this new technique’s technical feasibility, effectiveness, and safety in inducing transient middle cerebral artery occlusion in rats.

2. Materials and methods

2.1. Animals

Animal care and all experimental procedures were carried out in accordance with the guidelines of the European Communities Council Directive (86/609/EEC). Protocols were approved by the Ethics Committee for Animal Research of the local government. Twenty-four adult male Wistar rats weighing 350–400 g were used, of which two were subjected to angiographic anatomical study and technical pilot experiments, and 22 underwent transient MCA occlusion by the transfemoral selective intraluminal wiring technique.

All the animals were anesthetized with ketamine (50 μg/g i.m.), diazepam (2.5 μg/g i.m.), and atropine (0.05 μg/g i.m.), and maintained over a hot-plate at 37.0 ± 0.5 °C. Supplemental anesthetic doses were given as needed to maintain a uniform level of anesthesia.

2.2. Vascular anatomical study

In the preliminary anatomical study, complete global angiography was performed on one rat. The left femoral artery was surgically exposed and cannulated with a 24 G indwelling intravenous cannula (B. Braun Melsungen AG, Germany). Through the cannula, 4 ml Urografin (76%, Schering Inc., Germany) was injected to allow a complete angiogram to be obtained using a BV300 angiography system (Philips Inc., Netherlands) (Fig. 1A). On the other rat, selective internal carotid angiography was performed. The right common carotid, external carotid, and internal carotid arteries were surgically exposed. Following ligation of the external carotid artery, the common carotid artery was cannulated with a 24 G cannula, and 1 ml Urografin was injected to obtain the angiograms (Fig. 1B). The angiographic studies demonstrated that: (1) the aortic arch had three major branches—the innominate, the left common carotid, and the left subclavian arteries—similar to the case in humans; and (2) the projection of the pterygopalatine artery and branches of the external carotid artery in posterior-anterior view overlapped with that of the tympanic bulla, whereas only the internal carotid artery supplying the cerebrum extended medially to the tympanic bulla.

2.3. Focal cerebral ischemia by transfemoral selective "intraluminal wiring"

Transfemoral selective intraluminal wiring to induce MCA occlusion was performed in 22 rats. The left inguinal area was shaved and prepared steriley. The left femoral artery was surgically exposed and dissected. After ligation at the distal part of the dissected artery, the femoral artery was punctured and cannulated with a 24 G intravenous indwelling cannula. Systemic heparinization was obtained at the dosage of 150 IU/kg body weight. Through the femoral cannula, a 0.012 in. straight guidewire (Super floppy, Schneider Europe AG, Switzerland) was introduced into the femoral artery. Under fluoroscopic guidance, the guidewire was gently advanced retrogradely to the aortic arch. The basic maneuvers included pushing, twisting, and pulling the guidewire. All the manipulations were done gently under fluoroscopic guidance. As the guidewire is advanced from the femoral cannula to the aortic arch, especially in the portion of the abdominal aorta, it might enter one of the branches, such as the renal artery, celiac trunk and hepatic artery, or mesenteric artery. In such a case, the guidewire is pulled back a little to the aorta, and then pushed forward while being twisted in order to avoid the branches.

As a test of the technical feasibility of selective intraluminal wiring, the guidewire was successfully placed into the internal carotid artery on each side (Fig. 1C and D). In order to insert the guidewire selectively into the left common carotid artery, its tip was initially placed at the middle point of the aortic arch. The guidewire was then twisted anticlockwise. Immediately, the tip of the guidewire turned upwards, it was gently advanced to enter the left common carotid artery. In the neck, the guidewire passed upwardly and slightly laterally until the level of the first cervical vertebra, where it turned medially. By further slowly and gently advancing the guidewire, it progressed cranially, with its course medial to the left tympanic bulla (where the tympanic bulla was placed) as an important landmark in guiding this selective intraluminal wiring, until its tip occluded the origin of the left MCA (Fig. 1E). In addition, fluoroscopy was used with the head in lateral projection to check the proper position of the guidewire, showing it passing through the carotid canal and into the cranium (Fig. 1F). At this time, it is also feasible to advance the guidewire to the left anterior cerebral artery, which involves the occlusion of the origin of the MCA, because the tip of the wire can completely block retrograde blood flow from the ipsilateral anterior cerebral artery while the proximal portion of the micro wire at the bifurcation and internal carotid artery blocks blood supply to the MCA. If the guidewire was shown to be in some other artery, such as the left lingual artery (Fig. 1G and H), it was pulled back to the neck and another attempt was made.

The guidewire was left in place for 60 min, and then removed to permit 24 h of reperfusion. The left femoral artery was tied, the wound was closed, and the animal was allowed to recover from anesthesia in a chamber with room air at an ambient temperature of 37 °C. Neurological evaluation was carried out 24 h after ischemia. The rats were killed, and the brains were viewed under stereomicroscopy and inspected for SAH, followed by processing to detect necrotic areas in order to evaluate and
Fig. 1. Complete angiography by injecting contrast medium through the transfemoral cannula indicates that the anatomy of the aortic arch and its three major branches is similar to that in humans (A). Selective carotid angiography (B) highlights the middle cerebral artery, anterior cerebral artery, internal carotid artery, and branches of the pterygopalatine artery. Note that the internal carotid artery (arrows) extends medially to the tympanic bulla (*), whereas the pterygopalatine artery (arrowhead) overlaps with the tympanic bulla. Fluoroscopy shows the micro guidewire is selectively placed into the right internal carotid artery (C) and the left internal carotid artery (D). Fluoroscopy shows two anatomic positions of the guidewire's tip. (E-G) Posterior-anterior view. (F-H) Lateral view in each position. The tip of wire is placed in the left middle cerebral artery (E) and left lingual artery (G), respectively. Note that the guidewire goes through the carotid canal (arrows) into the cranium (F), but when it is in the left lingual artery (H), it overlaps with the medial margin of the tympanic bulla.
2.5. TTC-staining (2,3,5-triphenyltetrazolium chloride (TTC)-staining)

TUNEL and hematoxylin–eosin staining, DNA fragmentation analysis, and caspase-3 activity determination were included to analyze the mechanisms and characteristics of neuronal death in this model.

2.4. Neurological evaluation

The forepaw-outstretching test was used to examine and score neurological behaviors as described previously (Garcia et al., 1995). Briefly, the rat was placed at the edge of the table with the tail being held to maintain the hindlimbs in the air. When the rat was walking on forelimbs, the symmetry in the outstretching of both forepaws was carefully observed. A score of 3 was assigned when both forepaws were outstretched, and the rat walked symmetrically; a score of 2 when the right side outstretched less than the left, and forelimb walking was impaired; a score of 1 when the right side outstretched more than the left, and forelimb walking was impaired; and a score of 0 when the right forelimb did not move.

2.5. TTC-staining (2,3,5-triphenyltetrazolium chloride)

The brains (n = 10) were carefully removed from the skull and fixed in 4% paraformaldehyde. The brains were removed and kept in the same fixative at 4°C for at least 48 h. They were then embedded in paraffin, and cut into 10 µm thick coronal sections. The slides were alternately processed for H&E staining and for TUNEL technology, used for the in situ detection of DNA fragmentation, specific for the detection of features of apoptosis. The sections were treated as instructed (Cheng et al., 2002) with an in situ cell death detection kit, POD (Roche, 1684817). Apoptotic cells were observed under fluorescence microscopy or by using a Vector VIP substrate kit (Lopez-Sanchez et al., 2004) (peroxidase detection; Vector Laboratories, SK-4600). Sections were compared to the unaffected hemisphere, which was used as control.

2.7. DNA fragmentation analysis

Fragmentation of DNA was analyzed in three rats. Freshly dissected sections were homogenized and lysed. Genomic DNA was extracted and subjected to electrophoresis, stained with ethidium bromide, and viewed under UV light (Hockenbery et al., 1990).

2.8. Caspase-3 activity

In three rats, caspase-3 activity was detected with a previously described method (Samhan-Arias et al., 2004), using Ac-DEVD-pNA as colorimetric-specific substrate. Brain samples were frozen in liquid nitrogen just after dissection. They were then homogenized and lysed. After removing non-lysed material, caspase-3 activity was calculated spectrophotometrically (at 405 nm) using an extinction coefficient for p-nitroaniline of 10,500 M⁻¹ cm⁻¹, and Ac-DEVD-CHO as specific caspase-3 inhibitor.

3. Results

In all 22 rats, the guideewire was successfully placed to occlude the origin of the left middle cerebral artery without complications, and all the procedures of the intraluminal wiring, beginning with introducing the guideewire through the femoral cannula, were completed within a few minutes.

3.1. Neurological evaluation

All rats survived for 24 h after the MCA occlusion by transfemoral selective intraluminal wiring.

Contralateral forepaw-outstretching deficits were observed in all 22 rats: 12 rats were scored as 0 (54.5%) and 10 rats as 1 (45.5%). The corresponding 95% confidence level limits for the percentage of rats scoring 0 are 33.7–75.4%.

No subarachnoid hemorrhage was observed in any animal.

3.2. Infarction volume: TTC-staining

In coronal and transverse sections, we observed the infarct tissue circumscribed to the vascular area corresponding to MCA territory. Serious damage was detected in the cerebral cortex and the striatum of the ipsilateral hemisphere.

F. Sun et al. / Journal of Neuroscience Methods 149 (2005) 82–89 85

calculate the total infarction volume by morphometry using 2,3,5-triphenyltetrazolium chloride (TTC)-staining.
Fig. 2. Cerebral infarction following MCA occlusion. TTC-staining in coronal (A) and transverse (B) sections shows the lack of staining in the ipsilateral hemisphere. Scale bar = 3.5 mm.

Table 1

| Characteristics of the infarct volume (IV) and total volume of the affected hemisphere (TV) |
|-------|-------|-------|-------|-------|
|       | Mean  | S.D.  | Count | Minimum | Maximum |
| IV    | 168.86 | 7.041 | 10    | 159.220 | 179.860 |
| TV    | 709.014 | 17.670 | 10    | 687.780 | 742.160 |
| % IV  | 23.919 | 1.338 | 10    | 21.890 | 25.480 |
| IV/TV index | 0.238 | 0.013 | 10    | 0.218 | 0.255 |

Values obtained from the coronal (Fig. 2A) and transverse (Fig. 2B) sections are summarized in Table 1. The correlation study (Fisher’s r to z) (Table 2) showed that TV correlated negatively with % IV and the IV/TV index ($r = -0.737$; $p = 0.0126$; 95% C.I. $-0.933$ to $-0.199$ in both), and that IV correlated positively with % IV and the IV/TV index ($r = 0.918$; $p < 0.0001$; 95% C.I. $0.684$–$0.981$), but did not correlate with TV. These data are shown in Fig. 3 as simple linear regressions between IV and the IV/TV index ($p < 0.0001$), and between TV and the IV/TV index ($p = 0.0126$).

3.3. Characteristics of neuronal death

The results from the slides alternately processed for H&E staining and for TUNEL showed the structural differences between the affected (left) hemisphere in comparison with the control (right) side. As can be seen in Fig. 4, H&E staining
Fig. 4. H&E staining in coronal section (A) to illustrate the areas of ischemic damage in left hemisphere (LH) compared with control (right hemisphere: RH). Scale bar = 0.8 mm. The areas within the squares marked in (A) are shown at higher magnification in B (RH) and C (LH), to illustrate the pattern of cell destruction. Scale bar = 0.5 mm.

Fig. 5. TUNEL technology in a coronal section (A) showing neural cell death in apoptotic areas (peroxidase reaction) of the left hemisphere (LH), and no reaction in right hemisphere (RH). Scale bar = 0.5 mm. CPu: caudate putamen; FL: forelimb area of cortex; Par1: parietal cortex, area 1; VP: ventral pallidum; Pir: piriform cortex, observed in detail within the black frame (B) and under fluorescence microscopy, showing green stained cells (C). H&E staining (D) of the Pir: note the lower number of necrotic cells (arrows) compared with apoptotic cells. Scale bar = 0.1 mm. The agarose gel (E) shows internucleosomal DNA fragmentation in the affected hemisphere (LH), whereas the control hemisphere (RH) shows no significant DNA fragmentation. Left line (*) was loaded with GIBCO BRL 1 kb DNA extension ladder.
showed damaged tissue, characterized by a majority of either basophilic neurons or ghost neurons with no nucleus and loss of all cellular detail, as coagulative necrosis or cavitation with neuronal loss. The TUNEL technique (Fig. 5) showed neuronal cell death at the level of the caudate putamen, ventral pallidum, and from the forelimb area of the cortex to the piriform cortex. The use of these two techniques is an aid to discriminating the number of necrotic cells compared with the estimated number of apoptotic cells (Fig. 5A-D). Also, intermucosal/sonal DNA fragmentation was observed in the striatum/putamen (Fig. 5E), whereas the control hemisphere showed no DNA fragmentation, as is observed in the upper band of the intact, undigested, genomic DNA. Moreover, a nearly two-fold increase in caspase-3 activity (data not shown) was detected in the affected hemisphere relative to the unaffected (control) hemisphere.

4. Discussion

Animal models of cerebral ischemia replicating features of human neurovascular disturbance greatly improve our understanding of human stroke and are helpful in developing new therapies. The intraluminal thread model of MCA occlusion in rats is well accepted, although it has some limitations: lack of direct visualization to monitor the insertion of the filament and uncertainty in the location of its tip; absence of a standard procedure for the technique; and surgical invasion in the neck. Furthermore, excessive insertion of the filament may perforate the intracranial internal carotid artery resulting in SAH. Finally, the transfemoral selective intraluminal wiring technique offers a minimally invasive method without neck surgery. Proper manipulation of the guidewire can avoid blood vessel perforation and subsequent SAH. However, this resistance is not always perceivable. Occasionally, there is a clear resistance even though the middle cerebral artery is confirmed as not yet being occluded (Schmid-Elsaesser et al., 1998). The other method in the literature involves selecting the length of the filament to advance into the internal carotid artery. The reported length of filament varies from 17 to 22 mm, depending on animal strain and weight (Longa et al., 1989; Karibe et al., 1994; Soriano et al., 1997; Zarow et al., 1997). But SAH may occur before the filament is advanced to the recommended length. Moreover, SAH is attributed to the sharp and stiff tip of the filament easily perforating small arteries. To avoid this complication, the filaments have been treated rounding its tip by heat or sandpaper or covering it with silicone or poly-L-lysan (Kozuma et al., 1986; Longa et al., 1989; Kawamura et al., 1991a; Belayev et al., 1996). In a comparison of two different filaments, Schmid-Elsaesser et al. (1998) found that SAH occurred in 30% of rats with 3.0 filament, and in 8% with silicone-coated 4.0 filament.

Premature reperfusion and insufficient MCA occlusion is also commonly encountered in the intraluminal thread procedure (Schmid-Elsaesser et al., 1998). It was attributed to a slight dislocation of the filament resulting from the concomitant rise in arterial blood pressure. In animal experiments with this technique, when the filament is not inserted deeply enough—for instance, just caudal to the bifurcation of the anterior and middle cerebral artery—initially, ischemia can be achieved due to occlusion of proximal internal carotid artery, but subsequently reperfusion may occur by retrograde blood flow from the contralateral, ipsilateral anterior cerebral artery and bifurcation of internal carotid artery, to the presumably occluded MCA. The diameter and quality of the filament are critical in an MCA occlusion-inducing model. That the literature has various modifications of filaments for the intraluminal thread technique is indicative of the absence of a standard filament in inducing MCA occlusion. Kuge et al. (1995) compared two types of nylon monofilament in developing MCA occlusion in rats, and demonstrated that even if they were both 4.0, the exact diameter, tensile strength and extensibility were not the same, and the sizes of the induced ischemia size were significantly different.

In the intraluminal thread procedure, the common carotid artery, external carotid artery, and internal carotid artery are dissected, and ligation and cutting of the external carotid artery are needed. Since there are numerous important vascular and nervous structures in the area of this surgery, incidental or unavoidable damage to these structures may lead to any of several complications and eventual death. Even without serious complications, artery spasm can occur due to surgical manipulation, which makes insertion of the thread more difficult and has a potential impact on the outcome. Dittmar et al. (2003) studied external carotid artery territory ischemia in rats after intraluminal thread occlusion of the MCA and found that 49% of the rats with MCA occlusion suffered from ischemia involving temporal, lingual, and pharyngeal musculature. As ischemia impairs mastication and swallowing functions, food and water uptake is restricted, resulting in body weight loss and limiting neurological recovery.

The MCA occlusion model by the transfemoral selective intraluminal wiring technique described here has some notably interesting points. Fluoroscopy provides a direct real-time image to localize the guidewire and its tip. The guidewire can hence be clearly demonstrated to have wedged into the target vessels. This can effectively block blood to the MCA, thus avoiding insufficient MCA occlusion. In the present study, MCA occlusion was achieved in all the animals, with consistent neurological and pathological outcomes. Moreover, the safety of guidewires used in endovascular procedures (angiography or angioplasty) has been well established in interventional radiology. Proper manipulation of the guidewire can avoid blood vessel perforation and subsequent SAH. Finally, the transfemoral selective intraluminal wiring technique offers a minimally invasive method without neck surgery.

The authors also recommend the inclusion of systemic heparinization when creating a transient MCA occlusion model. Insertion of a filament or guidewire can initiate
platelet aggregation and thrombosis during MCA occlusion (Memezawa et al., 1992; Garcia et al., 1995), and leaving the filament or guidewire in place to occlude the blood flow for 60 min may increase blood clotting. In addition, in our experience, wedging the guidewire in the middle cerebral artery may lead to vessel spasm, as was indicated by a slight resistance when removing the guidewire following 60 min of occlusion of the artery in the rat. This may contribute to further thrombus formation after MCA occlusion if systemic heparinization has not been established. Therefore, systemic heparinization must be regarded as an important technical step in a transient MCA occlusion-inducing model in rats. In our current model, the TTC-staining showed, in the ipsilateral hemisphere of all the animals, that the induced infarct area and volume were consistently reproducible. We observed an increased IV together with an increased IV/TV index, indicating that IV increased according to the TV. Furthermore, the characteristics of cell death induced in this model allow one to establish differences between necrotic and apoptotic cells, suggesting the correspondence with the effects of ischemia and of reperfusion, respectively.

In conclusion, we have presented a new technique to induce transient middle cerebral artery occlusion in rats by transfemoral selective intraluminal wiring. The technique appears to be feasible, reliable, and safe, with the major advantage of minimal invasion. Hence, it should have great potential in pre-clinical research on the pathophysiology of cerebral ischemia, as well as on new therapeutic interventions. Furthermore, it may be extensible to such other areas as coronary, mesenteric, hepatic, and renal ischemia.

Acknowledgements

The authors thank Professor J. Usón, Scientific Director of the Minimally Invasive Surgery Centre (Cáceres, Spain) for helpful discussions. We wish to acknowledge the technical assistance of Sandra Pulido. This work was supported by grant nos. SCSS0449 (to V. G-M); 2PR03C029 and 2PR02A042 (to C. L-S) and 2PR03A060 (to C. G-M) from the Junta de Extremadura.

References