Middle Cerebral Artery Occlusion of Rats: Pathological and Neurological Evaluation of the Model

Sıçanlarda Orta Serebral Arter Oklüzyon Modelinin Patolojik ve Nörolojik Olarak İncelenmesi

NEVZAT KAHVECİ, TÜLİN ALKAN, ENDER KORFALI, KASIM ÖZLÜK

Departments of Physiology (NK, TA, KÖ) and Neurosurgery (EK), Uludağ University School of Medicine, Bursa, Turkey

Abstract: Eighty seven Sprague-Dawley rats were used to study the anatomy of the horizontal segment of middle cerebral artery (MCA) and infarction after occlusion of this vessel. We investigated the size and location of the lesion produced, and found a correlation between infarct size and neurological deficit. Fourty rats were used to determine the anatomical variations of MCA after intracardiac carbon black injection. Five major patterns of MCA were defined and two of them were major and constitued 88% of rats. In the experimental group (n:20) through a subtemporal burrhole MCA was exposed and after defining the anatomical variations of the artery according to our classification, MCA was coagulated 3-4 mm length from the origin of the lateral striate arteries to the inferior cerebral vein and divided. Control rats (n:20) underwent identical surgical procedures except for occlusion. Twenty-four hours after MCA occlusion, all animals were neurologically evaluated. In the experimental group, one rat scored 1, five rats scored 2, nine rats scored 3, five rats scored 4. On the third day after occlusion the brains were removed and stained with 2% 2,3,5 triphenyltetrazolium chloride (TTC). In each animal, the area of infarction was assessed and graded using computer analysis method. There were no rats grade I infarcts, 4 with grade II, 10 with grade III, 6 with grade IV. This study show that once the anatomical variations of the MCA and its branches in our strain of rats was determined, it was possible to achieve 80% grade III and IV infarcts.

Key Words: Focal cerebral ischemia, middle cerebral artery occlusion, middle cerebral artery variations, rat

Özet: Orta serebral arterin (OSA) horizontal segmentinin anatomik farklılıklarını göstermek ve oklüzyon sonrası iskemik lezyonların dağılımı ve infarkt alanı lokalizasyonu ve büyüklüğü ile nörolojik bulgular arasındaki korelasyon için 350-400 g ağırlığındaki SD sıçanlar (n:87) kullanıldı. Bir grup sıçana (n:40) OSA'ın anatomik varyasyonunu saptamak amacıyla intrakardiak siyah karbon enjeksiyonu yapıldı. Beş değişik OSA yapısı gözlendi ve ikisi major variyasyonu (%88) oluşturuyordu. Deney grubunda (n: 20) OSA subtemporal girişimle açıldı, sınıflandırmamıza göre anatomik variyasyonu belirlendikten sonra arterin lateral striat arter ayrımından inferior serebral vene kadar olan 3-4 mm uzunluğundaki kısmı koagüle edildi ve kesildi. Kontrol grubu (n:20) sıçanlara oklüzyon dışındaki tüm cerrahi girişimler uygulandı. OSA oklüzyonundan 24 saat sonra bütün sıçanlar nörolojik olarak değerlendirildi. Deney grubunda; 1 sıçanın skoru 1, 5 sıçanın skoru 2, 9 sıçanın skoru 3, 5 sıçanın skoru 4 olarak saptandı. Oklüzyon sonrası 3. günde beyinler %2'lik 2,3,5 triphenyltetrazolium chloride ile boyandı. İnfarkt alanları kompüterize analiz metodu ile tayin edildi. Grade I'de 0, grade II'de 4, grade III'de 10, grade IV'de 6 sıçan saptandı. Bu tür sıçanlarda OSA ve dallarının varyasyonunun tanımlandığı çalışmamızda, %80 oranında grade III ve IV infarkt alanı saptandı.

Anahtar Kelimeler: Fokal serebral iskemi, orta serebral arter oklüzyonu, orta serebral arter variyasyonları, sıçan

INTRODUCTION

The development of a reproducible and reliable animal model for cerebral ischemia would allow the study of pathophysiological changes that occur during and after the event. For an occlusion method to be optimal, it must produce a high rate of consistent, uniform, large infarcted areas. The rat is a widely studied, readily available animal that has been intensively investigated, and is preferred for cerebral blood flow studies. Numerous rat models of cerebral ischemia have been developed. These include bilateral carotid occlusion (33), an intracranial compression model that causes cerebral ischemia due to increased intracranial pressure (3,15), unilateral carotid occlusion plus hypoxia or hypotension (22,23), compression of the neck with a cuff (32), arterial microembolisation (29) and four vessel occlusion methods (26). While these models have been useful for examining various aspects of cerebral ischemia, they cause global ischemia rather than focal lesions and are not reliable for investigating the effects of various pharmacological drugs. For these reasons, researchers have adopted the technique of middle cerebral artery (MCA) occlusion described by Tamura et al. (34). The original method has been modified over the years and has become widely accepted as the main model for investigating of focal cerebral ischemia.

Recently the intraluminal thread model for MCA occlusion has gained greater acceptance (16). Numerous modifications have been reported in the literature which indicates that the technique is not yet standardized (10,12,13,16,17,19,27,31). Also, problems with subarachnoid hemorrhage and insufficient MCA occlusion are more common in the intraluminal thread model than the open craniectomy model (1,13,14,16).

Our early experience (30) with the model Tamura et al. suggested that it was not possible to achieve 100 % incidence of infarction through focal occlusion of the MCA, even if the site was proximal to the olfactory tract, as reported (34). Although vascular anatomy should be similar within the same strain, inbreeding can lead to the development of new variations of cerebral arteries in new-generation rats. With this in mind, we investigated inbred rats in our laboratory, focusing on variations in MCA anatomy and its effect on the production of uniform infaction site and size after arterial occlusion.

MATERIALS and METHODS

In this study, eighty seven adult male Sprague Dawley rats weighting 350-400 g were used. A group of rats (n:40) without any surgical procedures were used to determine the anatomical variations of MCA after intracardiac carbon black injection (5ml). After the rats' brain were removed, we carefully inspected the MCA and its branches using an operating microscope, and then drew them in detail. The relation between the MCA and the olfactory bulb and inferior cerebral vein were also noted. The experimental group of rats were anesthetized with sodium thiopental 30 mg/kg i.p. A tracheotomy was performed and the animals were ventilated (Harvard small animal ventilator) with 70% nitrous oxide, 30% O_2 mixture containing 1.5% isoflurane. A femoral artery and vein were catheterized to allow continuous monitoring of arterial pressure (Protocol PROPAQ 104) and repeated sampling of pO_2, pCO_2, Hct, pH and for fluid administration. A rectal temperature probe was inserted and the animals were maintained in the normothermic range (37.4±0.4°C) using a radiant heat lamp.

Once these procedures were done, the animals were placed in the supine position with their head turned to the left, 2 cm curved vertical incision was made starting midway between the left orbit and external auditory canal. An incision was made to the temporalis muscle and dissected from the subtemporal bone and reflected forwards. Under the operating microscope, temporo-mandibular joint and coronal process of the mandible were seen and infero-temporal fossa was exposed. The pterygopalatine artery was protected and mandibular nerve overlying on the pterygoid muscle was followed medially to the foramen ovale was seen. Using a high speed drill 3-4 mm diameter burr-hole was opened, 3 mm anterior and 1 mm lateral to the foramen ovale. The dura was incised with a fine needle and olfactory nerve and middle cerebral artery were located. Before the occlusion was carried out, we identified the middle cerebral artery and its branches. The rats were divided into 2 groups. Control rats (n:20) had all the surgical procedures except occlusion. In the experimental group (n:20) MCA was coagulated 3-4 mm length from the origin of the lateral striate arteries to the inferior cerebral vein and divided.

Twenty-four hours after the coagulation procedure was done, we evaluated the rats' neurological status using the system described by Menzies et al. (18). A scale of 0 to 4 was used to assess the motor and behavioral changes after the middle cerebral occlusion (MCAO). The test consisted of various maneuvers: First, the rats were suspended by the tail approximately 30cm above the floor and

their forelimb posture was noted. Normal animals extended both forelimbs toward the floor and they were assigned a score of 0. When the forelimb contralateral to the side of the MCAO was consistently flexed during the suspension and there was no other abnormality, the rat was scored 1. Rats were then placed on absorbent pads and they were gently held by the tail. If animals showed an apparent decrease in grip strength in the contralateral forelimb when pulled by the tail, then they were assigned a score of 2. Thereafter and while still being held by the tail, the rats were allowed to move freely and were observed for circling behavior. Rats that moved spontaneously in all directions but established a monodirectional circling toward the paretic side when given a slight jerk of the tail were scored 3. Rats that showed a higher clinical score also showed all features of the lower grades. This neurological evaluation was completed within a few minutes.

On the third day after occlusion, animals were killed a lethal dose of Na-thiopenthal. Brains removed immediately, a coronal slice was made 5 mm behind the frontal pole, and the tissue was immersed in 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma Chemical Company, England) in 0.9% phosphate buffered saline, incubated 37°C for 60 minutes (4,24) and placed in formaldehyde. After TTC staining infarcted brain was visualised as an area of unstained brick red. Sections were photographed using color film (Kodochrome ASA 100) and the infact areas were drawn by an investigator who was blind to the rat's group identity. The area of tissue necrosis or neuronal injury, which was unstained by TTC, was calculated using computer analysis method. The percent of brain tissue infarcted was calculated after extracting the area of infarction from the area of the entire coronal brain section. The infarcted areas were graded using the pathological grading score developed by Menzies et al. (18). Grade I; the smallest sized lesion and ranged from 8-10 mm^2, grade II; 11-28 mm^2, grade III; 32-60 mm^2, grade IV; the largest lesions ranged from 63 to 84 mm^2.

RESULTS

Seven animals of the total 87 rats used during the experiment died from various reasons between day 1 and day 3 after MCAO and excluded from the study.

There was considerable variability in the number and location of the branches of the MCA from its origin to its bifurcation. Several branches constituted the lateral striate arterial complex, which supplies blood to the basal ganglia. The pyriform artery usually originated several millimeters above the rhinal vein, and then made a right angle deviation from the main MCA trunk. The pyriform branch was seldom divided further and the area supplied by this artery appeared isolated from other major surface vessels. The temporal artery arose opposite to pyriform artery and ran along the temporal lobe and rarely anastomosing with the posterior rhinal branch. Frontal branch ran superiorly and anteriorly and often divided into several branches as it approached the midline. Parietal branch ran occipitally paralleling the midline and giving off multiple branches that covered much of the cortical surface and it divided more often than the frontal artery. The frontal and parietal branches approached to the anterior cerebral artery (ACA) and posterior cerebral arteries (PCA) respectively.

We observed the following 5 major MCA patterns: (Figure 1) Type A, with pyriform and frontal branches anteriorly and temporal branch posteriorly (45%). Type B, with both pyriform and frontal branches but lacking the temporal one (43%). Type C, with both frontal and temporal branches but lacking the pyriform one (2,5%). Type D, with double pyriform branches but lacking the temporal and frontal branches (2,5%). Type E, showed fenestration of the horizontal segment of MCA (2,5%). Type A, B (88% of rats) were the most common patterns seen in our Spraque-Dawley inbreed rat strain.

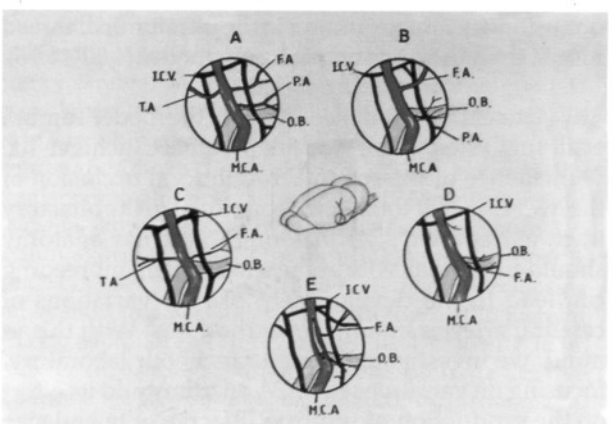

Figure 1: Five major branching pattern of MCA as seen through craniectomy and their relation to internal cerebral vein and the olfactor bulb. M.C.A., middle cerebral artery; P.A., pyriform artery; F.A., frontal artery; T.A., temporal artery; I.C.V., internal cerebral vein; O.B., olfactory bulb.

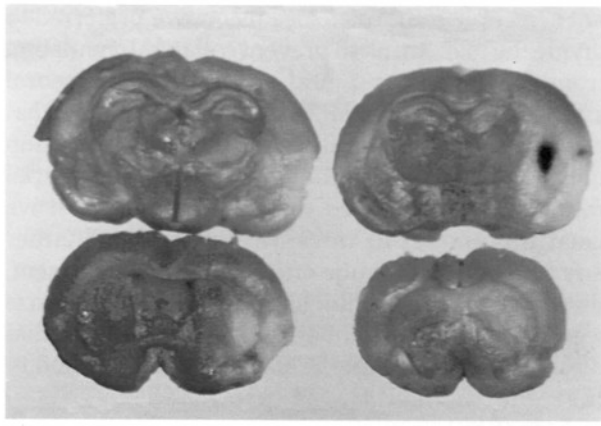

Figure 2: TTC-stained coronal section shows a large area of unstained infarct after MCA occlusion.

Twenty-four hours after MCAO, all animals were neurologically evaluated as described by Menzies et al. (18) None of the control animals showed any motor-behavioral abnormalities. In our study group, 1 rat was scored 1(5%), 5 rats were scored 2 (25%), 9 rats were scored 3 (45%), 5 rats were scored 4 (25%).

The size of infarction with TTC staining were graded as; 0 rats were graded I (0%), 4 rats were graded II (20%), 10 rats were graded III (50%), 6 rats were graded IV (30%) (Figure 2). In grade II, 1 rat had type C, 1 rat type D and 2 rats type B vascular pattern.

Significant correlation between the neurological scores and pathological grades were seen in our study (Table I).

DISCUSSION

Rats are convenient small animal model for study of pathophysiology and treatment of cerebral ischemia. Tamura et al. developed a subtemporal approach of proximal MCAO at a point near the origin of the lateral striate arteries, which produced infarction of both the cortex and the caudoputamen nucleus (34). The original technique, however, was very invasive and the animals survived only for a few hours. After some modifications such as preserving the zygoma and the masseter muscle, extended postoperative survival for several days and eventually the subtemporal approach became as the standard technique for producing focal cortical ischemia in rats (7,20,21,25).

However, in all of these studies cortical infarction size and location were variable. This variation has been attributed to variations in artery and to collateral circulation from the anterior (ACA) and posterior cerebral arteries (PCA). This led us to further investigate the artery itself especially the horizontal segment of MCA and it's branches. Rubino and Young observed six anatomical vascular patterns of the MCA in rats (28). In an other study, using high magnification in living animals, four major vascular

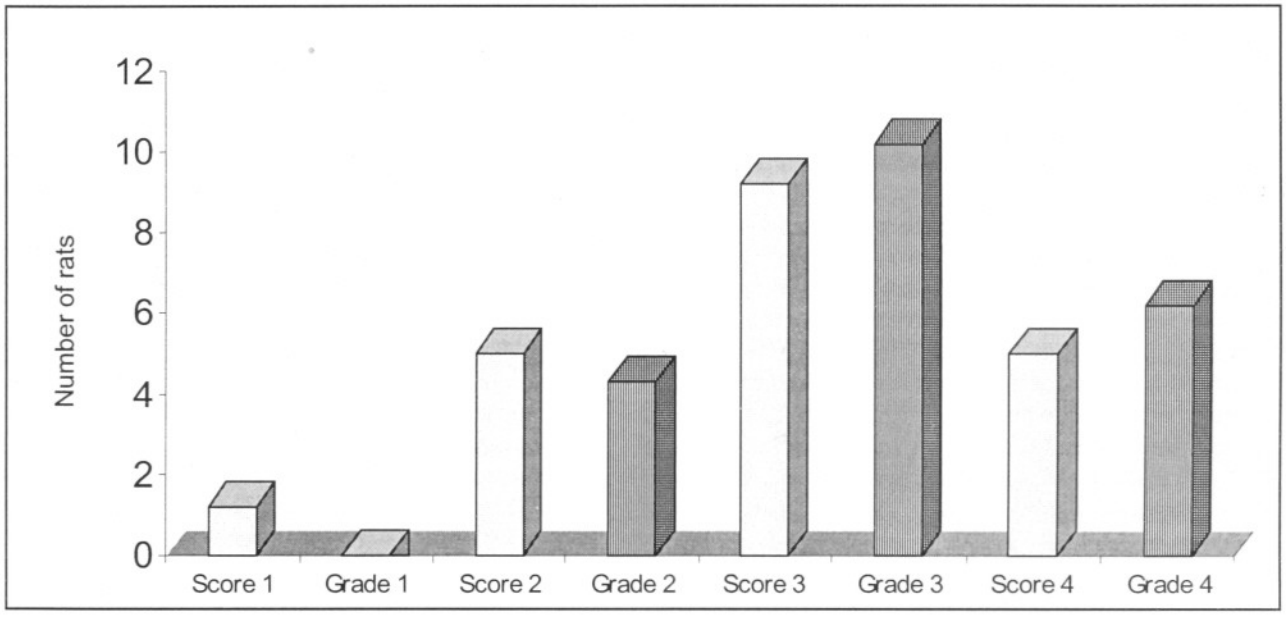

Table I: Shows correlation between the neurological and pathological grade after MCA occlusion.

patterns of the artery was demonstrated (18). In our study we identified five vascular patterns and two of them were major and constituted 88% of our rats. Type A (45%) and Type B (43%) resembled the Menzies et al.'s Type A (51%) and Type B (26.6%) findings, respectively. Although there was a wide difference between Type B, occurrence of Type A were almost equal. The remaining three patterns were either not observed or less than 2.5% in our study. Our Type B (43%) pattern resembled Type B of Rubino and Young's (28) and our type A (45%) is Type C (17%) respectively. Overall, only 43% of their rats' MCA pattern were similar to those we identified. Due to many variations of MCA, proximal occlusion below the rhinal fissure produced 13-67% lesions, whereas occlusion of larger lengths of the artery resulted in 100 % infarction rate (2). It was suggested that the latter model provided a more efficient compromise of the collateral blood supply from retrograde flow (18). In their model Menzies et al. (18) occluded the longer length of the artery and in addition the major MCA branches were also coagulated. In our study after determining the anatomical variations of the MCA and its branches in our strain of rats, and added the procedure of dividing the artery. By taking these steps, we were able to achieve 80% grade III and IV infarcted areas with less coagulation of the horizontal segment. The reason we chose not to use a larger approach to the MCA is that exposure of a larger area of the brain during craniotomy might alter blood-brain barrier permeability and intracranial pressure after infarction (5,11). Forsting et al., demonstrated in their study that craniectomy for cerebral ischemia in rats not only reduces mortality but also significantly improves outcome and reduces infarct size (9).

Using a similar occlusion technique, Menzies et al., observed considerable variability in their study and just over half of the infarct was characterized as large (18). The authors used two different type of coagulation in their study. In one experiment, the middle cerebral artery was cauterized from the lateral branch to the inferior cerebral vein. In the other which yielded more large infarcts, the artery was coagulated more extensively, including the main branchings for 7-9 mm distance. The authors claimed that more standardized and reproducible lesions were produced when a longer lenght of the MCA and its branches were occluded. In our study, using the similar tecnique with less segmental coagulation (3-4 mm) and dividing the main trunk after coagulation, produced grade III-IV lesions in 80% of the rats. Because coagulation alone occasionally is not accomplish complete occlusion, we preferred to divide the main trunk to prevent collateral circulation from the anterior cerebral and posterior cerebral arteries. El-Sabban et al., clearly demonstrated that electrocoagulation produced temporary occlusion only with embolization and in most cases partial recanalization followed the coagulation (8). If we used this model to interpret the basis of either infarction or therapeutic efficacy of drug treatment, there would be question as to whether the infarcts were embolic or thrombotic. Thus we thought that dividing the artery might eliminate the possibility that the ischemia was produced by distal embolisation.

The assesment of clinical neurological status of the animal accurately with stroke lesions is important for evaluation of the treatment. The clinical method of neurological evaluation after MCAO used by Bederson et al. (2) designates three neurological grades and Menzies et al. (18) lists four grades. According to Menzies et al. (18), in Bederson et al's grading system, the application of the test is sensitive only grossly paretic rats as also admitted by the authors. This is also noticed in our study and in our experiment Menzies et al.'s, grading system was used because it is applicable to even mild neurological deficits and also easily applied. For morphologic evaluation TTC staining and computer analysis methods are widely used for detection of lesions. TTC staining were shown as highly correlative with histological evaluations (24). Also 3 days after MCAO there is known to be an association between color differences between hemispheres and staining with TTC (18).

In our study the frontal and, indirectly, the pyriform branches were in collateral communication with the ACA, whereas the parietal and temporal branches collateralized with branches of the PCA. This explains why distal occlusion of MCA doesn't result in infarction. Cortical anastomoses are the key to the extent of infarction after a MCAO. This was clearly shown by the extent of the maximal lesions in the Menzies et al. study (18). In addition, as observed in our study, the margins of infarction never exceeded the sites of cortical interarterial anastomoses sites. Although it was stated by Menzies et al. (18), that none of the vascular anatomical patterns played a significant role in modulating the extent and severity of cortical ischemia, we found that the type E pattern, such as bifurcating or forking of the arteries may change the outcome of infarct. And also in our study in grade II size of infarction,

rats had rarer type of anatomical patterns such as type B, C or D.

Another important factor in infarction post-MCAO appears to be body weight, and, thus indirectly the age of the animals (18). In a study by Coyle (6) no young rats weighing below 150 g developed infarcts after proximal MCAO.

Defining of the variability in the anatomical patterns of MCA in the rats and also coagulation of the horizontal segment for at least 3 mm lenght plays very important role for success of the study. And also eliminating the rarer vascular patterns the incidence and size of infarction could be increased and well predicted large size of infarction with correlation of the infarct size with neurologic deficits could easily be achieved in our study.

Our results indicate that although Sprague-Dawley rats are the same strain due to their inbreeding the anatomy of the arteries considerable varies. So any investigator planning to use MCA ischemia model should establish normal anatomic pattern of their rats before starting the research.

Correspondence: Nevzat Kahveci
Uludağ University
School of Medicine
Department of Physiology
Görükle 16059 Bursa Turkey
Tel: 224 442 8855 Fax: 224 442 8034

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