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Middle Cerebral Artery Occlusion/Reperfusion Model in Monkeys

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Abstract

This study was designed to develop a reversible middle cerebral artery (MCA) occlusion/reperfusion model in monkeys.

By inserting a Cordis steerable guidewires balloon catheter (595-E014) from the femoral artery of 12 male adult monkeys (*Macaca mulatta*) weighting 5.5±0.5Kg, the balloon catheter could be inserted into the MCA and occluded the blood flow. Magnetic resonance T1, T2 and DWI showed ischemic signals in MCA supplied area. Neurological evaluation demonstrated neurological deficits in the ischemic group. Neuropathological examinations were consistent with the neurological evaluations. The results suggested that this monkey MCAO model seemed close to human stroke.

Keywords: Monkey; Middle cerebral artery occlusion; Balloon catheter

Introduction

Primate models of cerebrovascular disease have been found to be particularly useful in drawing analogies between observations in patients and in experimental models [1,2]. The anatomic territory of distribution of the middle cerebral artery (MCA) in human differs only slightly larger from that of other primates [3]. The neuropathology of MCA occlusion in human is comparable to that observed in the experimental MCA occlusion in primates [1,2,4]. The ideal model of focal cerebral ischemia has been focusing on the MCA.

Previously, the reversible primate models of MCAO were mainly the transorbital approach first described in 1970 [5]. Despite the reliability of this model being widely accepted in many laboratories [6-8] there are some disadvantages, including the involvement of meticulous orbital intervention, the possibility of injuring the nerve fibers normally found in the adventitia of MCA, and the potential CSF fistula. Manipulation of the vessel during surgical exposure and induced vasospasm may alter the efficiency of the subsequent collateral blood supply, leading to unpredictability of the resulting circulatory disturbance, and the unpredictability of the distribution and size of the lesion [9,10].

Considerable variability in the severity of ischemia exists even when surgical procedures are carefully standardized, and the physiologic parameters are kept as constant as possible [7]. The variable types of collateral connections that exist in individual animals possibly explain the enormous variability in the local circulatory conditions, characteristically observed after occlusion of MCA at the same site. This temporal and spatial circulatory variability makes the MCAO unsuitable for many of the statistical comparisons that are required in experiments attempting to predict the evolution of the lesion and to evaluate therapeutic interventions.

Therefore, this study was to develop a subhuman primate model of transient or permanent ischemia by varying the period of MCA occlusions.

Materials and Methods

Statement on animal welfare

Details of animal welfare and steps taken to ameliorate suffering are included in the methods section of the manuscript.

Adult male monkeys (M*acaca mulatta*) (5.5-6.0 Kg) were raised at Qingdao University Medical College and housed in groups of two in standard steel cages. The housing room was maintained on a 12:12 light/dark cycle, with lights on at 7:30 am. The temperature was kept at 25°C and relative humidity at 30%. All procedures were performed according to standards set by the Chinese Council of Animal Care and approved by the Animal Subjects Committee of People's Hospital of Ningxia

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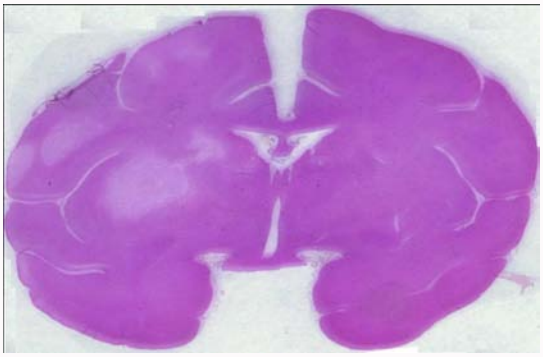

Figure 1: The representative photograph of monkey brain infarction at A17.5 section.

Hui Autonomous Region, Northwest University for Nationalities (approval IDSYXK(SU)2002—003). A total of 12 monkeys were used in this study.

MCA occlusion/reperfusion

This report described a method originally developed in rat [14], and adapted to the anatomical variables encountered in monkeys. 12 male adult monkeys (*Macaca mulatta*) were grouped into ischemia group and the sham group using a random digit table, with 8 in the ischemia group undergo the two-hour ischemia of the right MCA occlusion, 4 in the sham group at similar conditions, including the insertion of the catheter into the initial segments of MCA, but with balloons deflate.

The fasted monkeys were premedicated with Ketamine (4~5 mg/Kg, *im*), then under the 8% Chloral Hydrate anesthesia (150 mg/kg, *intraperitoneal injection, ip*). The rectal temperature was maintained at 37~38°C with heating lamps and a heating pad. The room temperature was kept at 25°C using an air-conditioner. The rectal temperatures, blood pressure, blood gas, blood sugar, were monitored during the experiment. Physiological variables were kept within the normal range. Plasma glucose level was normal in the fasted monkeys.

The monkey was positioned supine. Skin of the femoral artery area was routinely prepared. The balloon catheter was finally sent into the right MCA. Inflating the capsule of the balloon catheter occluded the MCA for two hours. The inflation was later released, and catheter removed. As shown in Figure 1, Cerebral angiography and MRI images of monkey were as follows: 1A, Cerebral artery branches; 1B, Steerable guidewire (arrow) and MCA; 1C, Ballon catheter (arrow) into MCA.

After acute experiments, all monkeys were sent to their cages with free access to water and food.

Arterial blood sampling and blood/gas analysis

0.5ml arterial blood sample was taken from the femoral artery before inserting the balloon catheter and analyzed with pH/blood gas analyzer (IL-1302, Instrumentation Laboratory system, U.S.A.).

Neurological evaluation

The monkeys were alert during the evaluation. With the monkey restrained in a primate restraint chair, the neurological evaluation was conducted before occluded MCA, 2 hours post reperfusion, and 24 hours following MCA occlusion/reperfusion. Particular attentions were paid to the level of alertness, spontaneous behavior, the deviations of the eyes and the head, response to the visual stimulation, spontaneous and evoked facial hemiplegia, proximal and distal limb power and tone. For all examinations, a modified relative simple grading scheme for neurologic deficit was used [6].

Brain Histopathology

After 24-hour reperfusion under 8% Chloral Hydrate (300 mg/kg, *ip*) anaesthesia, the monkey was perfused with 1000 *ml* 0.9% Normal Saline, then 800 *ml* 4% paraformaldehyde via the left ventricle with the descending aorta clipped. The brain was carefully removed and suspended in 4% buffered paraformaldehyde +20% sucrose for two days at 4°C, then transferred into 30% sucrose perfusion fixative for two days at 4°C.

The brain gradient desiccation and paraffin coating was as follows:

70% ethanol for two weeks; 80% ethanol for one weeks; 90% ethanol for one weeks; 100% ethanol for three days; Dimethylbenzene 1,2 for 45 min respectively; paraffin 1, 2 for two hours respectively.

The whole brain was then serially sectioned in a coronal plane into 6-micron sections with the microtome (Shouda, Japan). The sections were sequentially identified and stained with hematoxylin and eosin (*H&E*). Sections were examined sequentially to locate the tips (cauterized tissue) and to identify the characters of infarction. The brain slices at A17.5 Section which best indicated the ischemic changes of monkey MCAO, according to the monkey brain atlas [17], were scanned using the Leica Q500 IW image processing system (Leica, Germany) and the brain infarction areas were calculated.

Five view fields were selected in the ischemic regions for averaging. The residual cells in the core region of ischemia were accounted, and the residual cell ratio was calculated as the percentage of the cell density ipsilaterally/contralaterally.

Statistical Analysis

Statistical analysis was performed using SPSS 11.0 for windows. The results were subject to analysis of variance (ANOVA) for repeated measurements followed by unpaired Student-Newman-Keuls t-tests for inter-group comparisons of means and variances between groups. In all statistical analyses, a p-value of less than 0.05 was considered significant. All data are presented as mean±standard deviation of the mean (SD).

Results

The physiological parameters

The physiological parameters were shown in Table 1 for the sham and ischemia groups on animals during operation. Both groups were equivalent in regard to, the rectal temperature, the blood pressure, blood gases and pH. Physiological variables were normal. Plasma glucose levels were normal (3.5-6.2 mmol/L) in all fasted monkeys.

Table 1: Physiological parameters in monkeys subjected to 2h MCA occlusion.

	Sham (n=4)	Ischemia (n=8)
Rectal temperature (°C)	37.5±0.2	37.5±0.3
pO_2 (mmHg)	99.13±15.4	99.26±9.12
pCO_2 (mmHg)	37.56±6.13	37.24±5.31
pH	7.374±0.01	7.378±0.03
The blood pressure (mmHg)	127.8±12.8	126.1±11.2
The blood glucose	4.88±0.13	4.87±0.22

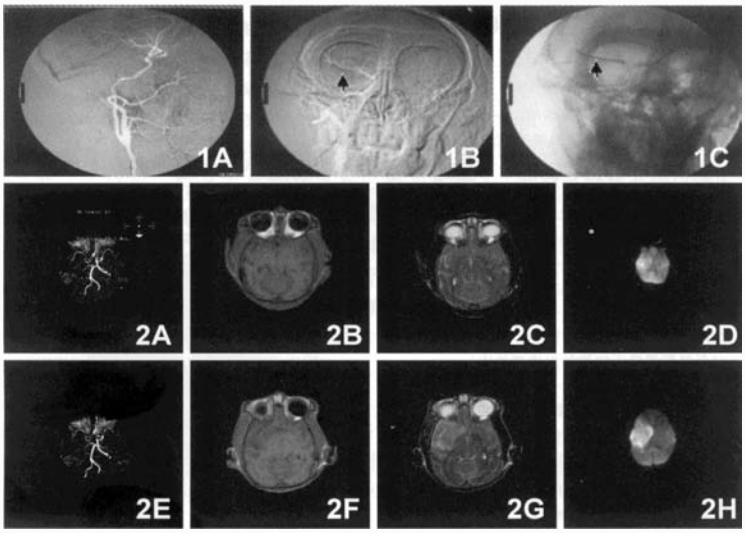

Figure 2: Cerebral angiography and MRI images of monkey.
1A: Cerebral artery branches; 1B: Steerable guidewire (arrow) and MCA; 1C: Ballon catheter (arrow) into MCA; 2A: MRA at 2h reperfusion; 2B: MRI T1 image at 2h reperfusion; 2C: MRI T2 image at 2h reperfusion; 2D: MRI DWI image at 2h reperfusion; 2E: MRA at 22h reperfusion; 2F: MRI T1 image at 22h reperfusion; 2G: MRI T2 image at 22h reperfusion; 2H: MRI DWI image at 22h reperfusion

Values were mean±standard deviations. Blood gases and pH measurements were obtained before inserting the balloon catheter.

Neurological deficit evaluation

MCA occlusion and reperfusion in primates produced a characteristic deficit similar with that in human stroke [7]. Focal neurological deficit signs such as hemiplegia and tonic eye deviation were observed immediately after occlusion of MCA in monkeys. The upper and lower extremities apraxia, weakness or hemiplegia; lethargy or coma could be found during the experiments in all cases of occlusion.

The neurological assessment chart [6] was used; There 0 indicates death, 100 indicates normal. The neurological deficit score was 38.75±10.31 at three hours after reperfusion, and decreased to 31.22±11.09 at 23 hours after reperfusion significantly ($p< 0.001$).

Histopathological changes were typical of ischemic infarcts. Infarctions were located in the cortex, the basal ganglia. There were no histopathological signs of infarction in ACA territory in all the cases because of the special anterior communication artery in monkey. The infarction areas at A17.5 Sections were 59.197±12.869 mm^2 as shown in figure 1. The swelling of the brain was found in the insular hemisphere.

H&E stain slice (bottom) was converted into white-black image using Leica Q500 IW image processing system. The white part was ischemic region at caudate and cortex of the experimental side in ischemia group.

Most of the neuron was eumorphism with the clear nucleus at the unaffected side of the cortex and caudate nucleus. In the ischemic cortex, the gray matter was involved, and the white matter relatively spared. Neuron loss manifested at the core region of the striatum at the ischemic side with the residual neuron had an incomplete cellular structure. Neuron loss in the peri-ischemia of insular cortex was not clearer than that in the striatum. The cell density in the insular striatum group was less than that in the control group. The cell density in the insular striatum was less than that in the control side. The sham operations produced no pathological evidence of cerebral infarction.

MRI findings

Brain infarctions were detected in all monkeys following 2 h MCA occlusion. After 22 h post-reperfusion, the infarction territory enlarged gradually. The middle line of brain shifted to the left even brain herniation at 22-hour post-reperfusion. Figure 2 displays the MRI and MRA images at 2 h, 22 h after reperfusion.

The white areas indicate increased brain density in T_2 images and show the regions of ischemic changes at the right basal ganglia and cortex.

Discussion

This study shown that in monkeys with an occlusion and reperfusion of MCA, typical hemiplegia and brain infarctions could be produced by controlling the balloon catheter.

Proximal MCA occlusion in all animal species results in severe reductions of lCBF within the striatum [11,12]. It is therefore, not surprising that the earliest signs of ischemic damage are frequently detected in this structure [8,9,13]. The extent of infarction or ischemic cell damage produced by 1h of MCA occlusion in the cat or monkey was reported to be small, consisting of scattered foci of necrosis or ischemic cell damage [8,9].

Based on the anatomy of the monkey cerebral artery, the anterior cerebral artery and the anterior common communication artery are unique. Therefore, it is easy to advance the catheter into the MCA.

In addition to giving rise to a consistent ischemic lesion, this MCA occlusion had several important advantages. First, the catheter is very thin, and the balloon is inflated at the proximal segment of MCA for two hours, so that the blood vessel can avoid being damaged. Second, the occlusion can be disrupted at predetermined post-occlusion times, providing the opportunity of reperfusion at any time to produce variable degrees of lesions which is suitable for experimental purposes. Third, direct manipulation of intracranial arteries can be avoided without damaging the dura. Finally, reperfusion can be made simply by letting off the air of the balloon, thereby, facilitating chronic survival.

The present method is somewhat similar with that of Longa [14]. However, the blunt round tip of Dermalon surgical suture in Longa method is unchangeable; and therefore, cannot fit all kinds of diameter of blood vessels, leading to the lesion variable at some extent [15,16]. The catheter balloon takes advantage of the Dermalon surgical suture to produce occlusion of MCA in a manner of *all or none*. So the lesion's degree is dependent on the period of occlusion. In our observation 2 h occlusion produced typical hemiplegia, brain infarction at the right basal ganglion and cortex confirmed both by neuropathological study and MRI.

Therefore, transient occlusion of the MCA was achieved in a manner resembling aspects of human ischemic stroke and recanalization of an occluded cerebral artery. The periods of occlusion can be transient or permanent according to experimental design; easily controllable by deflation of the balloon or not. Techniques used in this method seemed much simpler than those used in transorbital or intraorbital approaches.

Detailed chronic pathological correlation will be the subject of a subsequent study.

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