

Estimation of the Infarct Size on Occlusion of the Middle Cerebral Artery in Primates

K.S. SATHEESHA, M.S. SOMESH, SHAKUNTHALA R PAI, GIRISH V PATIL

ABSTRACT

Introduction: The model of single artery occlusion in subhuman primates was studied, to verify whether there is any correlation between micro-circulatory perfusion impairment and area of infarction after occlusion of middle cerebral artery and to find out whether there is any definite “reperfusion window” which could be used effectively to prevent or reduce the area of infarction.

Materials and Methods: For the present study 24 healthy adult monkeys of either sex were procured and the Middle cerebral artery (MCA) was occluded and reperfused for given set of time periods and the infarction size of the brain was determined.

Results: The percentage of the infarction size after occluding the blood vessel increased considerably, as the time interval prolonged. After the reperfusion of the arteries for shorter intervals like 30 min, 4 hours and 12 hours, there was a significant decrease in the infarct size, whereas there was no effect on the infarct size after 12 hours.

Conclusion: In the regions of focal cerebral ischaemia, the adverse effect of focal ischemia may be minimized if, in the acute phase, functional microcirculation is increased by some therapeutic intervention aimed at augmenting blood flow through the ischemic region.

Key Words: Cerebral Ischaemia, Occlusion, Reperfusion, Infarct.

INTRODUCTION

Cerebral ischaemia, usually of the Middle Cerebral Artery (MCA) territory, is one of the common causes of cerebrovascular diseases. The human syndrome of stroke consists of the abrupt development of a focal neurological deficit whose origin can be traced to either the occlusion of a cerebral vessel (usually arterial), or to the spontaneous rupture of an intracranial artery, with a consequent haemorrhage in the brain parenchyma or in the subarachnoid space [1]. The retrospective analyses of a large group of stroke patients have revealed that a vast majority were afflicted with one of the 3 anatomical lesions. About 75% of all the stroke affected cases are the clinical expression of brain infarctions and large, usually single, brain haemorrhages constitute the background for about 11% of the strokes and primary, non-traumatic, subarachnoid haemorrhages make up almost 5% of all the strokes [2-4]. Brain infarction is a localized destructive lesion which owes to the occlusion of a brain vessel, usually an arterial one. On the basis of long term, repeated physiological and clinical observations, the model of a single artery occlusion in subhuman primates has been found to be the closest to an ideal model of ischaemic stroke [5]. The clinical and the morphologic features of this model are very similar to those of massive ischaemic stroke (brain infarction) in human cerebral hemispheres [6].

The transorbital surgical approach to the initial segment of the MCA was first described in the 1970s [7]. The reliability of this method to induce either an infarction or tissue abnormalities that evolved into an infarction has been confirmed in many laboratories around the world [8,9]. This method allows reperfusion of the ischaemic territory, an important pre-requisite in the development of experimental methods of transient ischaemic attacks. An extensive injury to the neurons precedes the development of microvasculature obstruction and neuronal destruction which is more widespread than impaired perfusion [10]. Different experimental

approaches have shown that under physiological conditions, all the brain capillaries are perfused continuously with the plasma [11]. Under the ischaemic conditions, the swelling of the astrocytes and the capillary endothelial cells or a destruction of the micro-vascular basal lamina occurs during the first hours of the ischaemia, resulting in capillaries with narrowed or total occluded lumens [12]. This results in a decrease in the capillary perfusion velocities and later, in the non-perfusion through the capillaries. The aim of the present study was to verify whether there was any correlation between the micro-circulatory perfusion impairment and the area of infarction after the occlusion of the middle cerebral artery and to find out whether there was any definite “reperfusion window” which could be used effectively to prevent or to reduce the area of the infarction.

MATERIALS AND METHODS

For the present study, 24 healthy adult monkeys (*Macaca radiata*) of either sex were procured from non-forest areas with prior permission from the forest Department, Government of Karnataka, India. They were maintained in the animal house of Kasturba Medical College, Mangalore, Manipal University, India, till the experiment was done and they were maintained accordingly after obtaining the ethical clearance for the present study. Anaesthesia was induced by giving intravenous injections of Nembutal (pentobarbital) (25mg/ml of distilled water for the injection/kg) and it was maintained in a supplementary, minimum dose as and when it was necessary, through the peripheral leg vein, by using a butterfly needle initially.

After the scarification, the disposals of the animals were carried out through an environment friendly incinerator which was maintained by our university hospital.

a) Surgical Methods:

Under aseptic conditions, the femoral vein and artery were exposed

and they were canalized with a 18 G polyethylene tube for the intermittent infusion of the anaesthesia (whenever it was required) and for the blood pressure recording. The anaesthetized animal's head was fixed firmly in a stereotaxic head holder. The right eye was enucleated and the orbital contents were removed to expose the bony orbit through a transorbital approach. With the help of an operating microscope, the lateral wall of the orbit was widened by using a high speed electric dental drill. The dura matter was cut and the animals were divided in to 3 groups as control, permanent occlusion and temporary occlusion, with 8 animals in each group.

Group I - Control Animals

Group II - Permanent occlusion

Group III - Temporary occlusion

In group I, the MCA was freed from the arachnoid matter and it was exposed; in group II, the MCA was occluded with an aneurysm clip permanently for the given time periods; and in group III, the MCA was temporarily occluded and it was removed after different time periods of the occlusion. The reflow was established and 1½ hours, 4 hours, 12 hours and 24 hours were chosen as the time periods of the study. The dural opening and the orbit were sealed with dental cement to prevent a CSF leak. The whole procedure was carried out under aseptic conditions and the animals were given an antibiotic coverage of 250mg bd penicillin. A periodical blood gas analysis was done and the Blood Pressure (BP) was recorded periodically to ensure that the animals were maintained under normal physiological conditions. The body temperature was monitored with a rectal thermometer and it was maintained within normal limits by using a heating pad (*).

b) Quantification of the ischaemic area:

At the end of the experimental period, the brains of the animals were quickly removed and they were sliced with improvised slicer at the levels of the optic chiasm, the temporal pole and the mammillary body. The slices were incubated at 37°C for 15 minutes in a 1% solution of TTC in saline. The reaction was terminated by substituting the incubation medium with 10% formalin [13]. By this method, the normal area was stained red, while the ischaemic area was left unstained. The ischaemic area was quantified with a locally improvised grid in millimeters (mm²). At the end of the selected time period, for the study, the animals were heparinized and perfused with 10% buffered formal saline, after cutting the superior vena cava. Once the animals were properly formalin fixed, the formalin was washed off with warm saline, followed by a treatment with India ink in gelatin, till the animals turned black [14]. After keeping them overnight at 4°C, their brains were removed for the quantification of the perfusion defect. An improvised grid was used for the quantification. The total infarct size was determined by TTC (mm²) in millimeters after the occlusion and the reperfusion of the blood vessel for various time intervals, viz 30 minutes, 4 hours, 12 hours and 24 hours and these values were compared with those of the control groups. The statistical analysis of the data which was obtained was done by using the SPSS software, version 6 and the Student's t test was applied for the comparison between the groups. A p value of ≤ 0.01 was considered as significant.

RESULTS

(A) Physiological parameters: Various physiological parameters like the rectal temperature, blood pressure, etc were recorded for the primate animals, before and 4 hours after the cerebral occlusion, as has been shown in [Table/Fig- 1], as a part of the

routine protocol.

(B) The cerebral infarction: In the brains of the control animals (group I), there was no evidence of an infarction, as the blood supply was not interrupted and the brain of these primates looked relatively healthy [Table/Fig-4].

The percentage of the infarction size was determined by TTC (mm²) in millimeters in the permanent occluded group (group II) after occluding the blood vessel for various time intervals, viz 30 minutes, 4 hours, 12 hours and 24 hours. As was expected, the infarct size was found to increase considerably as the time interval was prolonged, as has been shown by the arrows in [Table/Fig- 5 (a) and (b)]. The values of the infarct size were also determined, which were significant when they were compared with those of the control animals for the same given time intervals [Table/Fig- 2],[Table/Fig-3].

	Before occlusion	4hr after occlusion
Rectal temp (°C)	38.0 ± 0.3	37.9 ± 0.2
Blood pressure (mmHg)	126 ± 18	120 ± 28
PCO ₂ (mmHg)	45.1 ± 12	50.6 ± 82
PO ₂ (mmHg)	162.2 ± 08	154.4 ± 02
pH	7.42 ± 0.01	7.40 ± 0.02

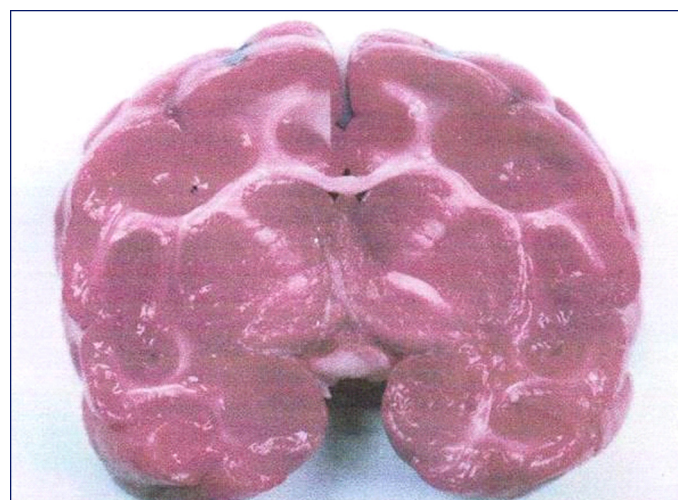
[Table/Fig- 1]: Physiological parameters in a primate model of focal cerebral ischaemia

Groups	Time after MCA – occlusion (h)			
	0.5 h	4 h	12 h	24 h
1. Control	0.0	0.0	0.0	0.0
2. Permanent occlusion	5.90 ± 1.18***	9.70 ± 2.15***aa	11.23 ± 2.89***bb	13.48 ± 3.13***ccde

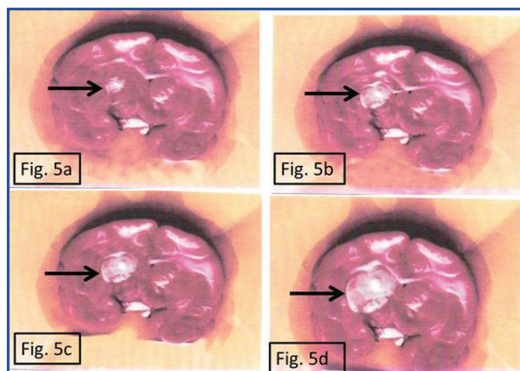
[Table/Fig-2]: Percentage of infarction size determined by TTC (mm²)

Reflow (h)	Group – 3		
	Control	4h – occlusion	12h - occlusion
0.5	0	8.55 ± 1.86***	11.20 ± 2.95***
4	0	7.60 ± 1.81***	11.23 ± 2.89***
12	0	7.45 ± 1.78***	11.23 ± 2.89***
24	0	13.48 ± 3.13***aabc	11.23 ± 2.89***

[Table/Fig-3]: Percentage of infarct size after restoration of blood supply



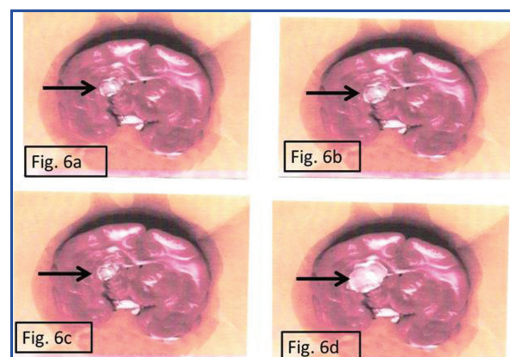
[Table/Fig-4]: Coronal Section of the cerebrum of control animals.



[Table/Fig-5]: Sections of cerebrum of Group II primates, where the arteries were occluded for various time intervals, viz, a = 30 min, b = 4 hrs, c = 12 hrs and d = 24 hrs.

*** $p < 0.001$ Control v/s Experiment

aa $p < 0.01$ 0.5 h v/s 4 h
 bb $p < 0.01$ 0.5 h v/s 12 h
 cc $p < 0.01$ 0.5 h v/s 24 h
 d $p < 0.05$ 4 h v/s 24 h
 e $p < 0.05$ 12 h v/s 24 h



[Table/Fig-6]: Sections of cerebrum of Group III primates, where the arteries were occluded for 4 hrs and reperfusion was done for various time intervals, viz, a = 30 min, b = 4 hrs, c = 12 hrs and d = 24 hrs.

*** $p < 0.001$ Control v/s

Experiment

aa $p < 0.01$ 0.5 h v/s 24 h
 b $p < 0.05$ 4 h v/s 24 h
 c $p < 0.05$ 12 h v/s 24 h

In the group III animals, after the occlusion of the arteries for 4 hours and 12 hours, the reperfusion of the arteries was done for various time intervals, viz 30 minutes, 4 hours, 12 hours and 24 hours respectively and the changes in the infarct size were determined. In the brains with the 4 hrs occlusion and reperfusion for 30 minutes, 4 hours and 12 hours, – the infarct size was found to be decreased [Table/Fig-6(a)and(c)] and the values were significant as compared to those of the controls, whereas the reperfusion for 24 hours had no beneficial effects [Table/Fig- 3]. Also, in the occlusion of the arteries for 12 hours with reperfusion for various time intervals, there were no changes in the infarct size [Table/Fig-3].

DISCUSSION

The ability to determine the location and the extent of the areas of infarction in the cerebrum after ischaemia is essential for the assessment of medical or surgical interventions for the confirmation of the clinical findings and for the evolution of new diagnostic techniques. The traditional histopathological methods which are based on the staining with Hematoxylin and Eosin can accurately and reliably distinguish the infarcted from the normal tissues. But the infarction is identified during its microscopic examination by its shrinkage, by the dark staining of the neurons and by the swelling

with vacuolization of the perineural and the perivascular glial elements [15,10]. The shrinkage of the tissue during its preparation for the microscopic examination may affect the estimate of the size of the area of the infarction. Originally, [2,3,5]- Triphenyltetrazolium hydrochloride (TTC) was used to test the viability of seeds [16] and since 1958, it has been used as a stain to detect the ischaemic infarction in mammalian tissues [17]. While this water soluble salt is not a dye, it is reduced by certain enzymes in the normal tissues to a deep red, fat soluble, light sensitive compound (formazan), that turns the normal tissues into a deep red, thereby clearly delineating the abnormal areas [16].

Depending on the physiological factors that affect the cerebral tissue [15], ultra structural changes may appear after 15 to 30 minutes of the ischaemia [18], as the rate of the cerebral oxygen consumption (CMRO2) is reduced in the immediate post ischaemic period, as was demonstrated in the rat brain [15]. Staining with an agent such as TTC, that is based on the presence of an intact enzyme electron transport chain, might be expected to delineate the infarction at an earlier stage than the traditional histologic methods. The TTC staining is an excellent research method that can be used to confirm the size and the location of the areas of the infarction, and it may be valuable in determining the presence and the location of the infarction in autopsy samples.

Local blood flow changes are brought about by the modification of the micro-circulation in physiological and pathological states [19]. In focal cerebral ischaemia, a reduction in the blood flow has been well documented in the clinical practice [20,21]. A morphometric study of the functional microvasculature was performed on ischaemic cats [22] and also in asphyxiated [23], hypoxic [24], haemorrhagic [25] and hypercapnic rats [11].

Since it was difficult for the study to delineate only the patent micro-vasculature, the India ink perfusion method was adopted. A transcardiac infusion which was done by using the animal's own systolic pressure at the beginning of the perfusion, avoided the errors of the technique such as variations in the infusion pressure, size of the cannula, and partial obstruction of the tip of the cannula against the wall of the vessel [26]. The perfusion mixture was prepared just before its use, to prevent the possible variations due to the differences in its viscosity.

Also, there is a lot of literature on cerebral ischaemia. In a study, when the focal ischaemia due to the Middle Cerebral Artery Occlusion (MCAO) was induced in halothane anaesthetized hypertensive rats, the most marked initial impairments in the perfusion were observed in the core MCA territory and a secondary perfusion impairment was found to develop over time in the peri-focal region [27]. The changes in the interstitial oxygen tension (pO_2) in the cerebral ischaemic regions, particularly in the ischaemic core and the peri-focal area, induce a complex series of molecular pathways which involve the signaling mechanisms, gene transcription, protein formation, etc and free radicals and oxidative stress have been suggested to be involved in each of the steps in the injury cascade [28]. Also, cerebral ischaemia, followed by the vessel ischaemia/reperfusion of the MCA was studied and it was noted that it alters the vessel properties of the brain arteries in rats, thus inducing an inflammatory response and excessive generation of the reactive oxygen species, leading to an increase in the wall thickness, the cross-sectional area and the wall/lumen, and decreased wall stress, which were prevented by the

treatment with CR-6, a vitamin-E analogue, that is an antioxidant and a scavenger of the nitrogen-reactive species [29]. Also in another similar study, the administration of oral CR-6, immediately within 2 hours of ischaemia of the MCA occlusion, resulted in the reduction of the glutathione consumption and the superoxide generation in the ischaemic brain, with a decrease in the infarct volume and an ischaemia induced neurological deficit, thus protecting the brain from reperfusion injury but not against permanent ischaemia [30].

CONCLUSION

So in conclusion, in the regions of focal cerebral ischaemia, where the blood flow is deficient, as was demonstrated in the present study, the adverse effect of focal ischaemia can be prevented or minimized, if in the acute phase, the functional microcirculation is increased by some therapeutic intervention which is aimed at augmenting the blood flow through the ischaemic region.

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