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Original Research Article

OCCLUSION OF MIDDLE CEREBRAL ARTERY CAUSING THE CEREBRAL EDEMA IN PRIMATES, A STUDY BY SPECIFIC GRAVITY METHOD

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ABSTRACT

Aim: To check if there is any relation between the compromise of microcirculatory perfusion, infarct location and edema of cerebrum after occluding the middle cerebral artery and to find out if any reperfusion remedy, which can be applied to minimize the infarction and cerebral edema.

Materials and Method: The present study included 24 adult monkeys which were procured from the non-forest areas. The cerebral edema was assessed by the specific gravity method.

Results: It was observed that, after the permanent occlusion of the MCA for 30min, 4 and 12h, there was insignificant risein the quantity of water of the cerebral hemisphere, when compared to sham operated monkeys. There was no contralateral variation in the water content with the occlusion of one cerebral hemisphere for 30min, 4,12h. At 24h, there was an insignificant increase in the contralateral cerebral hemisphere.

Conclusion: We opine that, it is the quantity of the infarct rather than the occurrence or absenteeism ofthe reperfusion, which is responsible for the development of cerebral edema. This study supports the clinician opinion, which reports that the brain edema will not trigger after the thrombolysis and recanalization of the obstructed artery.

KEY WORDS: Cerebral edema, Ischemia, Specific gravity, Perfusion, MCA.

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INTRODUCTION

Cerebral edema, a life threating pathological condition occurs following head trauma, cerebral ischemia and hemorrhage. The management of cerebral edema is very important because of the increased mortality in the young generation because of the traumatic brain injuries. There areresearch being performed using the animal model to study the mechanism of the formation and management of cerebral edema. The corticosteroids and hypertonic saline have given good results in managing the cerebral edema

[1-3], but the satisfying effects are inadequate because these drugs can't remove the basic factors. Theycannot be used for longerdays due to their side effects. Since the etiopathology of cerebral edema is convoluted, the appreciative of the complete mechanism of the formation of cerebral edema is requiredto develop the anti-edema drugs. The cerebral edema management is intricate, and the best results can be expected only if the diagnosis and management are offered in a timely situation [4]. In the present study, a model of single artery occlusion in subhuman primates was studied to verify if there is any relation between the compromise of microcirculatory perfusion, infarct location and cerebral edema.Themiddle cerebral artery was occludedto find out if there is any definite reperfusion remedy, which can be used to reduce the infarction and cerebral edema.

MATERIALS AND METHODS

The present study is an animal model research which included 24 adult monkeys which were procured from the non forest areas. The monkeys were of both the sexes and the age the monkeys were unknown. The monkeys which had diseases were not included in the present study. The cerebral edema was assessed by the specific gravity method. The middle cerebral artery of the brain was blocked to study the cerebral edema. The heads of caudate nucleus and insula were dissected out from the ischaemic brains of a group of monkeys. The specimens were cut into many small pieces and weighed. Their density was estimated by floating them in auninterrupted gradient column, which was prepared by using kerosene and bromobenzene. The tissue samples were compared with the standards (K_2SO_4) of known density to check the specific gravity.

The method of preparing the gradient column was modified and standardized. Bromobenzene and Kerosene were mixed in a definite ratio. For the specific gravity of 1.0350, 4.2ml of bromobenzene and 8.25ml of kerosene and for specific gravity of 1.0650, 4.8ml of bromobenzene land 7.7ml of kerosene were mixed. The lighter mixture solution (1.0350) was carefully layered over the denser mixture (1.0650) which was placed in a 25ml graduated cylinder. The solution was mixed with a soft copper wire coiled at one end to prepare the gradient. Mixing was begun midway of the column at the junction of two solvents. Short strokes extending about 1cm above and below the junction of the two mixtures were used. In this manner mixing distance was increased until the entire column was mixed.Tissue samples and standards which accumulate in the cylinder are removed with a net made of fine-mesh nylon stocking sewn to a loop at the end of the wire. After a series of sample measurements, tissue sample and standards are collected by raising the net. The gradient remains nearly constant if movement during removal and insertion of the net is slow enough to prevent visible mixing of solvents. The gradient was always standardized just prior to the measurement of specific gravity of a series of samples.

The K_2SO_4 was used to prepare thestandards and they were dried for one over night at 100^0C temperature. The quantity of K_2SO_4needed to prepare the required sp. gr. standards can be found in the Handbook of chemistry and physics. The quantity (grams per liter) of K_2SO_4 needed to prepare the generally used specific gravity standards are as follows: 1.0325 (4.10), 1.0364 (4.65), 1.0406 (5.19), 1.0447 (5.74), 1.0489 (6.28), 1.0530 (6.83) and 1.0572 (7.39). A standard curve was prepared from their position in the gradient mixture after placing the duplicate volumes (5-15µl) of each standard in the column. The position of a standard or sample was determined 5 min later using graduated scale on the cylinder after keeping it over the column.

Statistical analysis was done utilizing the students 't' test for small samples. ANOVA test was also performed since the sample size was small. Patency of the right MCA following clipping was ascertained by India ink perfusion.

RESULTS

After the standards were added to the gradient column, the drops descend rapidly at first, become nearly stationary at 2 min. Brain samples also drop rapidly initially but remain stationary at 5 min comparing with the standards tissue sample's sp. gr. were recorded.There was significant change observed between control

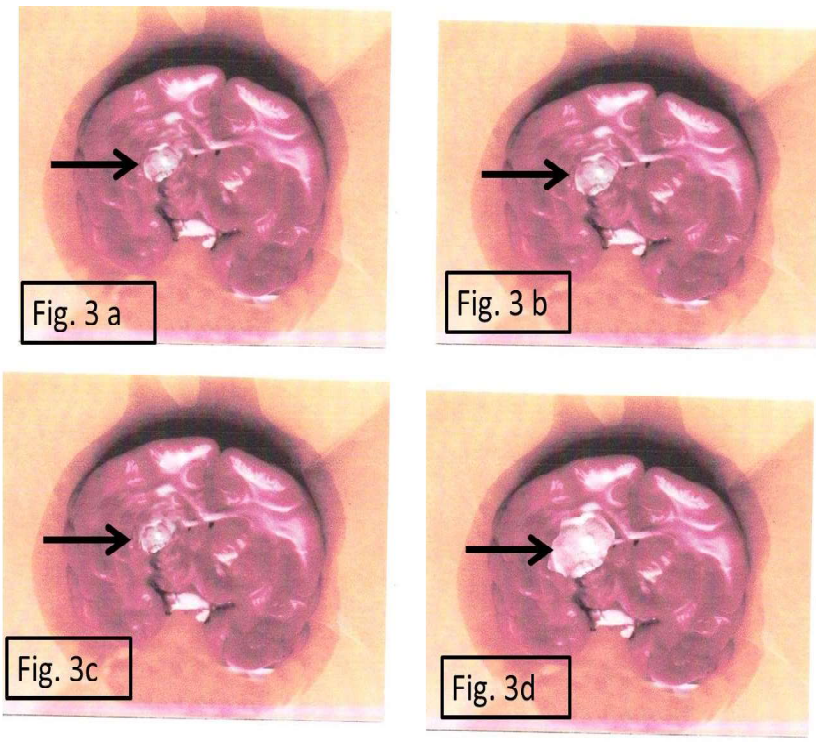

Fig. 1: Sections of cerebrum of primates, where the arteries were occluded for 4 hrs and reperfusion was done for various time intervals, viz, a = 30 min, b =4 hrs, c = 12 hrs and d = 24 hrs.

Table 1: Specific gravity of brain tissue values is mean ±S.D for 6 animals.

	SAMPE TIME (h)				
	Control	0.5h	4h	12h	24h
Permanent occlusion	1.05±0.001	1.051±0.001	1.048±0.001^{aa}	1.045±0.002^{bbdd}	1.036±0.013^{cceeff}
4h occlusion followed by reflow	1.05±0.001	1.047±0.01	1.047±0.001**	1.047±0.001**	1.046±0.001**
12h occlusion followed by reflow	1.05±0.001	1.045±0.01	1.045±0.001	1.045±0.001	1.045±0.001

and experiment group (Fig. 1) and also between 0.5 V/s 4h, 0.5 V/s 12h, 0.5 v/s 24h, 4h v/s 12h, 4h v/s 24 and 12h v/s 24h.The table 1represents the specific gravity of the brain tissue values.

DISCUSSION

Since the tissue sp. gr. is dependent upon the sp.gr. of the tissue solids as well as water content, sp. gr. of tissue solids was determined in the infracted area. Sp. gr. of tissue solids was not affected by brain edema resulting from water intoxication, therefore a change in water volume of tissue could be calculated from a change in tissue sp. gr.The factors of high sensitivity, ease of measurement, and the use of small samples also make the method desirable for the measurement of cerebral edema. Since simplicity and speed in the determination of brain edema could be used to advantage both in the laboratory and operating room, studies were carried out to evaluate the use of a density gradient for brain water measurement.

The extent of cerebral edema formation and the sequel of reperfusion on a time line have not been previously reported. The clinical reports based on computed tomography evaluation opine that the ischemic stroke is autonomousi-rrespective of its reperfusion status. It was also described that the differentiation of an infarct and cerebral edema is hard in a computed tomogram scan. The mass effect due to the infarct can only be an indirect method of measuring the cerebral edema and this can be detected only with the large sized infarcts.In the present study, we used a focal ischemic model in the primate model. There was no change in the infarct or edema observed between the permanent occlusion and 4 hours of temporary occlusion. The data of the present study indicate that a brief period (30 min) off focal ischemia exhibited a minimal increase or no increase in the cerebral water content.

The trans-orbital of the MCA was adopted to avoid contact with the brain and to minimize

surgical trauma. The water content of the entire hemisphere was estimated to avoid the risk of missing maximal edematous areas when dissecting regions. Cerebral edema in middle cerebral artery occlusion wasstudied in primates by wet weight: dry weight method [5]. The increase in water content seen after 12h, may perhaps be due to tissue necrosis, rupture of cell membrane, accumulation of lactate, lowered cerebral energy needed to regulate water imbibition across the cell membrane, increase in arachidonic acid, other free fatty acids.The sudden increase in water content on re-establishing blood flow through the occluded artery could be due to the increased permeability of the cell membrane to water and ions and the increased pinocytic activity in the endo thelial cell layer. Ischemia has been shown to release nor-epinephrineand 5HT which result in an increase in CAMP facilitating phosphorylation of membrane proteins and changes in the permeability of the cell membrane. The increase seen prior to 12h of reflow following 4h of occlusion could perhaps due to electrolyte and water movement from the blood into the brain without protein extravasation. A similar increase in water content on restoration of blood flow has been reported earlier [6]. This increase in water content can result in increased tissue pressure which will affect the local tissue perfusion in addition to increased compression of microvasculature [7]. In monkeys with right MCA occlusion, a progressive reduction in capillary luminal dimension was observed [7]. Unchecked edema could result in larger infarcts or continued increase of intracranial tension, resulting even in coma and death.

Increase in water content in the contralateral hemisphere has been observed by earlier workers, but only after about six or twenty four hours of ischemia. No astrocytic swelling in the opposite hemisphere has been observed in ischemia [8]. In our study, there was no Evans blue extravasation over the contralateral hemisphere may be due to changes in neurotransmitters and CAMP level or neurogenic mechanisms [9].

The mechanism underlying the increase in water content of the contralateral hemisphere in focal ischemia has to be taken into account in any study of edema.

Since the tissue sp. gr. is dependent upon the sp.gr. of the tissue solids as well as water content, sp. gr. of tissue solids was determined in the infracted area. Sp. gr. of tissue solids was not affected by brain edema resulting from water intoxication, therefore a change in water volume of tissue could be calculated from a change in tissue sp. gr. The factors of high sensitivity, ease of measurement, and the use of small samples also make the method desirable for the measurement of cerebral edema. Since simplicity and speed in the determination of brain edema could be used to advantage both in the laboratory and operating room, studies were carried out to evaluate the use of a density gradient for brain water measurement.In our previous report, we opined that the zones of focal ischaemic lesions and its effects can be controlled in an acute situation by providing the microcirculation by giving some medical intervention [10].

CONCLUSION

It is understood from the present investigation that, microcirculation because of the therapeutic intervention can minimize the focal ischemia in an acute phase.It is obvious that the brain edema because of ischemic stroke is having significant relation with the quantity of the infarct and not with the status of reperfusion. The findings of the present study supports the clinician opinion that aggravation of brain edema will not happen after the destruction of the thrombus or artery recanalization.This information may help us to more accurately select those patients who should receive thrombolytic and neuroprotective therapies.

Conflicts of Interests: **None**

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