The Production and Clinical Features of a Chronic Stroke Model in Experimental Primates

BY LINDSAY SYMON, F.R.C.S., N. W. C. DORSCH, F.R.C.S., AND H. A. CROCKARD, F.R.C.S.

Abstract: The characteristics of stroke in baboons produced by transcranial occlusion of the middle cerebral artery were studied by clinical examination and serial cinematographic studies, the animals being maintained for three years following the stroke. The characteristic deficit in all animals was an initial, fairly dense facio-brachial weakness with, in a few instances, some accompanying leg weakness for a few days, rapidly improving over the first few months. Some animals retained very evident arm weakness; most animals retained weakness of the face; the majority showed recovery of reaching and placing reactions and some movement in all joints of the upper limb, although fine movements of the fingers remained invariably impaired. Homonymous hemianopia, at least to attention, also appeared to be characteristic, but all of the animals recovered a normal gait and leaping was regained. The close correlation between this and human stroke appears to confirm that middle cerebral artery occlusion in the baboon is a reliable, repeatable and acceptable stroke model.

Introduction

With the development of reliable clinical methods for the assessment of regional circulation in the brain,1-3 and increasingly available clinical13-18 and experimental19 analyses of the metabolism of neurons in normal and ischemic circumstances, there have been recurrent attempts to establish an effective regimen which, applied early in the development of ischemic cerebrovascular disease or following an acute stroke, might minimize a neurological deficit and diminish the mortality.5-12 The demonstration in experimental circumstances of early swelling and raised local pressure following a stroke,13-14 of disordered vascular reactivity and diminished autoregulatory potential,6-18 and of a quantitative relationship between the preservation of neurological function and cerebral blood flow (CBF)17 gives clear indication for optimism in the treatment of cerebrovascular disease. Debate and disagreement, however, still bedevil the field, probably in part because of imperfect definition of the extent and density of ischemia produced in the experimental models used.

The present investigation was undertaken to provide a clinical correlate for the standard acute ischemic insult which has been subjected to prolonged investigation in this laboratory: acute middle cerebral artery (MCA) occlusion in the baboon. We supposed, from the work of others,18-20 that such a lesion would parallel more or less closely the typical features of acute stroke in man, and that by varying the degree of involvement of the perforating vessels21,22 a varying density of neurological deficit could be achieved.

Methods

Ten baboons (Papio cynocephalus), weighing between 14 and 26 kg, were used in the study. After sedation with phencyclidine (Sernylan), anesthesia was induced by a small dose of thiopental (3 or 4 ml of a 25 mg per milliliter solution) administered by catheter into the short saphenous vein. Endotracheal intubation with a cuffed tube was carried out and anesthesia maintained with pure oxygen containing halothane in a concentration varying between 0.5% and 1%. Intermittent positive pressure ventilation was used, the respiratory efforts of the animal being controlled by gallamine triethiodide (Flaxedil) (in an initial dose of 1 mg per kilogram), and the stroke volume adjusted to produce a Pco2 between 25 and 30 mm Hg; in two animals there was some difficulty in lowering the Pco2 to the desired level. One femoral artery was exposed and cannulated for continuous recording of blood pressure by a femoro-aortic catheter. Intermittent samples of arterial blood were taken from this catheter for analysis in a micro-Astrup apparatus for pH, Pco2, and Po2. No attempt was made to lower the blood pressure artificially, although the smallest animal in the series (which subsequently died) became appreciably hypotensive under this standard anesthetic regime.

An oblique incision upward and backward from the zygoma just behind the outer angle of the eye was carried in...
CHRONIC STROKE MODEL IN PRIMATES

the same line through the temporal muscle to expose the groove on the outer aspect of the skull at the outer end of the sphenoidal wing. A small one-half inch trephine disk was raised immediately above and behind the outer end of the wing, and a craniectomy made of the outer end of the sphenoidal wing and a semicircular area about one inch in size into the subfrontal and anterior temporal regions. The craniectomy was carried as close as possible to the base of the skull. The dura was opened through a semicircular incision just behind the outer end of the sphenoidal wing, with a re-entrant incision along the line of the sylvian fissure. This triradiate exposure enabled the dural flaps to be held back satisfactorily with stay sutures, and the MCA then was approached by gentle retraction of the tip of the temporal lobe upward and backward over lintine strips. With controlled ventilation, the extent of retraction necessary was quite minimal. The medial end of the sylvian fissure was opened, the terminal carotid, anterior and middle cerebral vessels identified, and the MCA clipped between two standard McKenzie clips. The outer clip was allowed to lie on the segment of the MCA bearing the perforating vessels, since it was desired to produce a fairly dense neurological deficit. In one animal, the anterior choroidal artery was accidentally damaged and included in the proximal clip (this animal had a large infarct and subsequently died), and in two further animals a determined effort was made to doubly clip the MCA very close to its origin, completely sparing the perforating segment. In no instance was the MCA clipped distal to the segment bearing the perforating vessels.

The dural flaps were laid loosely over the brain and covered with a piece of Surgicel. The muscle was closed in two layers with continuous catgut sutures, and the closure completed with a subcutaneous running suture of catgut and interrupted black silk to the skin. The black silk sutures were removed at the end of a week. No dressings were necessary. The animals invariably breathed spontaneously on cessation of the halothane anesthesia and elevation of the blood pressure. The animal had a dense infarct and impaired consciousness postoperatively. It was also unusual in showing a distinct leg weakness even at ten days, and at this stage tinct leg weakness was still evident at three days and distinct drowsiness was still evident at three days.

Results

Two of the animals in the group died postoperatively. One of them (a 14-kg animal, the smallest in the series) had an entirely uncomplicated operative course, although the blood pressure fell during surgery to a much lower level than was encountered in the rest of the group. No attempt was made to support the animal postoperatively. It was also unusual in showing a distinct leg weakness even at ten days, and at this stage progressive deterioration in consciousness was followed by the animal’s death. Considerable swelling of the hemisphere, with a shift of midline structures, was evident and the infarct was clearly very large. The second death occurred in the largest animal of the group (a 26-kg baboon) in whom the anterior choroidal artery was occluded in the proximal middle clip. We had intended to observe in this animal whether or not this would appreciably increase the deficit and, indeed, the clinical picture was that of a fairly severe stroke, although it would have been impossible to differentiate the clinical picture from those of the more severe, purely middle cerebral occlusions. The picture was unfortunately complicated by the development of a secondary wound infection in this animal, which caused its death at nine days postoperatively.

The remaining eight animals made an uninterrupted recovery from surgery, and were maintained in captivity for three years following the production of the stroke. They had a fairly typical clinical course, and the details of each animal’s progress are given in table 2. Over the first 24 to 36 hours the animals were generally quiet; they could be handled without anesthesia and a limited neurological examination was possible, provided the approach was invariably made from the side of the hemiparesis. In all instances, a dense facial weakness was evident and neurological recordings were made with the animal in its cage or, if sufficiently tractable, with the animal led into a closed room. During this period of time, one or two tube feedings of protein concentrate in milk were given under tranquilization with phencyclidine. It was unnecessary thereafter to feed the animals artificially. From time to time over the first four months, the animals were taken from their cages into an enclosed run, and serial cinephotography of their free-ranging activities made to assist in the detailed analysis of the neurological deficit. This is of particular value where a comparative analysis of deficit density is being undertaken; different observers can see the same length of film recurrently and a much more detailed analysis is thereby possible. From about four months onward, however, the facilities available for free-range observation of the animals were clearly inadequate. The animals had returned to completely normal aggression and activity, and observation without anesthesia was completely impossible. The stability of the deficit thereafter could be assessed only by cage observation.

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*Postoperative death.
Flow Immediately After MCA Occlusion (From Symon et al.19)

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this, as a rule, persisted up to three years, although tone appeared to recover in a number of the animals after about four months. It was possible, however, to observe that food, for example, would be retained much longer in the cheek pouch on the paralyzed side (fig. 1), suggesting that complete muscle tone was never regained in any of the group. The animals invariably showed some degree of hemianopia, which persisted over the entire period of observation. It is impossible to state with confidence how far this represents an inattention defect and how far it represents complete loss of the visual half-field, since the testing was restricted to the reaction to menace, as shown in figure 2. However, evidence of this could be obtained in all the animals as late as three years, although the clinical testing without anesthesia of a substantially recovered 20-kg baboon leaves much to be desired. In all the animals who recovered, leg weakness was minimal and evident only in the first day or two. Thereafter, when the animals were observed free-walking, which was possible in the majority up to three or four months, it was almost impossible to detect any weakness of the leg, and the animals would leap to a height of 3 to 4 feet without difficulty and remain quite straight.

In the first few weeks after operation, when unrestrained, the animals tended to circle. Within the first three to four days this circling movement was in some instances almost continuous, suggesting the description of forced circling19 already observed in hemiplegic dogs and monkeys. Later on, however, the circling became a much more casual affair and appeared to arise only from the visual inattention. The animal would walk straight if its attention were fixed but, when walking along a corridor, it would tend to rotate as its attention became drawn to something in its retained visual field (fig. 3). It did not appear, therefore, that forced circling was a characteristic of any of the animals over a prolonged period of time, although a tendency to circle during walking could be seen up to four months in many of the group. From four months onward it was impossible to allow these animals unrestrained movement, and the judgment of the presence or absence of circling thereafter was very difficult. The activity of the animals could be well determined by the presence or absence of leaping when unrestrained. Characteristically, the animals, when allowed free from their cage within the confines of a run, would rush from one end to the other leaping toward the heavily barred windows. This enabled the judgment of the use of the paralyzed hand, and only the most severely affected animals showed an absence of leaping lasting more than a few days.

The hemiparesis produced by MCA occlusion with involvement of the perforator bearing segment was usually fairly dense in the arm. Although complete abolition of arm movements, even in the first few days

FIGURE 1
Animal 4, one week postoperatively, showing a dense facial weakness while feeding.

FIGURES 2a AND b
The difference in the reaction of an animal to menace in the inattentive half-field and in the normal half-field, two months after surgery.
Following surgery, was rare, it occurred in the two animals who died and in two of the fairly densely affected survivors. From ten days onward, however, all animals showed reasonable movement in the proximal muscles of the arm and evident recovery in the muscles of the elbow and wrist (fig. 4). The differentiation of stroke density in the group could be performed best by considering the time scale of recovery of finger movements and of the reaching and placing reactions. Although these could not be tested in detail, the recovery of use of the forelimb could be well assessed even on cage observation by observing finger movements in reaching and placing. None of the animals recovered a completely normal forelimb. The least affected (Animals 6, 8, 9 and 10) would, by four months, hold onto bars as they leaped in free-range, and movement of the fingers was evident on cage observation. The limb, however, was in no instance used as freely as its fellow, and only in Animal 6 was it possible to watch the animal for more than a few minutes and be uncertain from its upper limb movements which was the hemiparetic side (fig. 5).

From four months on, the available neurological examination did not really change.

**Discussion**

The results reported clearly demonstrate that the technique of acute MCA occlusion with involvement of the perforating vessels will produce a clinically recognizable stroke in the large experimental primate. The facial weakness, the semiflexed resting position of the elbow, wrist and fingers, and the impairment of joint position sense so obvious in placing reactions closely parallel the features produced by a classical stroke either in degenerative vascular disease or in MCA stenosis associated with aneurysmal surgery. The model is clinically ideal. Other investigators**19** had reported disappointing correlation between the size of the infarct and the clinical deficit produced. Although it is true that in our group a fairly uniform general picture has been evident, detailed pathological examination after perfusion fixation, which was carried out by Dr. J. B. Brierley of the Medical Research Council Laboratories, Carshalton, and whose findings will be published subsequently, has indicated that the single...
An animal with little deficit eight months after surgery (Animal 6). The animal is climbing using the hemiparetic side and gripping with the hemiparetic arm, and during climbing weight-bearing is on the affected hindlimb.

animal in the group in which perforating damage was clearly excluded had a clinical course significantly less severe than that of the group as a whole. Although the picture remained similar in qualitative terms, the rapidity of recovery of elbow and finger movements and the placing reactions were appreciably quicker in this animal than the others in the group. In the same way, the animals in which the infarct was significantly smaller than the group as a whole — although once again the qualitative stroke seemed closely comparable — in general showed a more rapid recovery than those in which a very large infarct had been produced. It is striking, however, that in the group as a whole the major portion of ischemia fell upon the basal ganglia. The distribution of the infarct mainly involved the basal ganglia and the lips of the sylvian fissure (the opercula). The cortex observed from the lateral aspect of the brain, apart from the immediate temporal pole, would have passed for normal.

Although no attempt was made in our group to modify the degree of infarction by lowering the blood pressure, our experience in Animal 4 would appear to confirm the views expressed by Thompson and Smith. The extent of this infarct was large and the animal did not survive. This corresponds with studies reported elsewhere by us, in which acute ischemic swelling in baboons could be produced only by simultaneous occlusion of the terminal carotid and middle cerebral vessels during a period of hypotension. In the present study, the addition of anterior choroidal occlusion to MCA occlusion clearly increased the infarct size but, unfortunately, in this animal the study was cut short by its premature death. It seems, however, that a stroke of a density varying from inattention in the contralateral forelimb, a moderate facial weakness, inattentive hemianopia and impaired placing reactions at the least, to extensive weakness of the upper limb with gross impairment of finger movement, may be produced by varying the extent of division of the perforating vessels in association with a main MCA occlusion. When more severe brain swelling is desired in the model, then simultaneous occlusion of the anterior choroidal and middle cerebral arteries or of the MCA and terminal carotid (which is substantially the same thing) would appear to be necessary. The addition of hypotension could be regarded as a valuable adjunct.

It is clear, of course, that the more severe the ischemic deficit produced, the higher the mortality will be of the experimental group. In view of the extreme expense involved in the production of such a model, a high yield is essential. In our present group, an 80% yield would appear to be satisfactory, but there are clear grounds for believing that only occasional mortality need be expected if segmental occlusion of the MCA alone is performed at normal blood pressure, and that this will produce a reliable and recognizable ischemic stroke. Modification of the model to enable selective occlusion of perforating branches to the basal ganglia alone is also possible if required.

References
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