Hemodynamic and Clinicopathologic Verification of a Stroke Model in the Dog

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SUMMARY Twenty-five mongrel dogs had intracranial internal carotid and proximal middle cerebral artery occlusions. The animals were followed for one week and subsequently sacrificed. This method of clipping produced a mean drop in cortical cerebral blood flow of 48.4% as measured by the $^{18}$Kr washout technique. Cerebral blood flow was not affected by the brain retraction necessary for clip placements. Mortality in the first week was 16% and neurological deficits were observed in 73% of the animals. Infarction was present in 80% of the animals, and the mean percent infarction of the affected hemisphere was 17.00 ± 3.98SE.

This is a useful stroke model in an animal which is easily available, inexpensive, and suitable for microvascular intracranial surgery research.

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AN OBSTACLE in stroke research is the lack of a suitable animal model. Primate models have been generally preferred because of their similarity to humans and the relatively high infarction rate with various methods. On the other hand, primates have become expensive, and, because of their small size, unsuitable for microvascular bypass research. The dog is an easily available, relatively inexpensive experimental animal, and is suitable for cerebral microsurgery. The main disadvantage has been the difficulty in producing infarction due to the great amount of collateral circulation the dog has available. For this reason, the only feasible way to induce cerebral infarction in the dog is to occlude the vasculature distal to the major collateral circulation. Embolization techniques have been utilized aimed at occluding the horizontal portion of the middle cerebral artery. A problem with these techniques is the occasional fragmentation of the embolus or its extracranial passage.

The other possibility involves the use of intracranial vascular clipping. This can be done either via a transorbital approach or utilizing direct retraction of the brain to approach the internal carotid artery and its branches. Because of our interest in microvascular bypass research, and the measurement of cerebral blood flow with direct cortical exposure which requires a sufficiently large area of craniectomy to expose also the internal carotid intracranially, we decided to seek a model of intracranial vascular clipping through the direct approach. We selected a 2 clip method via a pterional approach which gives a high infarction frequency, but still allows passage of the radioisotope into the ipsilateral common carotid artery to the clipped hemisphere.

This paper describes the technique and its effects on regional cortical cerebral blood flow (rCBF), neurologic deficit, and infarction.

Materials and Methods

The experimental series consists of data from 36 mongrel dogs weighing 15–18 kg. Eleven dogs served as unaltered controls (sham-operated) and 25 had intracranial vascular occlusion (untreated-ligated). All the animals were sedated with phencyclidine...
HC1 1 mg/kg i.m. and given atropine sulfate 0.02 mg/kg. Each animal was also given a prophylactic dose of Combiotic (Pfizer) 2 ml i.m. The animals were paralysed with gallamine triethiodide 2 mg/kg i.v. with supplemental doses as needed, intubated and passively ventilated with a Harvard animal respirator on 60% N2O-40% O2. The respiratory rate and tidal volume were adjusted to maintain an end tidal CO2 of 5%. All operative sites were infiltrated with 1% procaine HC1. The femoral artery was catheterized to measure arterial blood pressure and to collect blood for gas analysis. A catheter was placed in a forepaw vein to administer drugs and fluids. A third catheter was placed into the right lingual artery and passed retrograde into the common carotid artery for the injection of 8Kr.

Head surgery was performed as follows: the right temporalis muscle was subperiosteally dissected and reflected over the zygoma. A craniectomy was performed over the Sylvian fissure and was carried inferolaterally removing as much of the sphenoid wing as possible. The dura was opened and the frontal lobe slightly retracted to expose the internal carotid artery. With the aid of a Zeiss operating microscope, the arachnoid around the vessels was dissected off and the internal carotid artery distal to the posterior communicating artery, and the proximal middle cerebral artery were occluded with Yasargil microclips (fig. 1). In the 11 sham-operated dogs the vessels were exposed and dissected free of arachnoid, but not occluded.

Arterial blood gases were measured frequently with an IL 113 blood gas analyzer and maintained within physiologic limits by respirator adjustments and sodium bicarbonate i.v. as needed; pH 7.30–7.40, PO2 80–120 torr, and PCO2 38–42 torr in all animals. Arterial blood pressure was monitored continuously with a Sanborn pressure transducer and recorded in a Sanborn 350 physiograph along with the end tidal CO2. Mean arterial blood pressure was maintained at 115 ± 10 torr by the use of phlebotomy, intravenous saline and a tilt table.

Regional cortical cerebral blood flow (rCBF) was measured by the 8Kr washout technique using an intracarotid injection of 3–6 mCi of the isotope dissolved in saline. A Geiger-Müller tube was placed over the exposed right hemisphere, and its output was amplified and recorded on a Harshaw count ratemeter and strip chart recorder. The blood flow was calculated by the height/area method. rCBF was measured prior to ligation (Initial-rCBF), immediately after ligation (Postclip-rCBF) when applicable, 3 hours after ligation (3 hour-rCBF), and one week later (1 week-rCBF). In addition, rCBF was measured after cerebral retraction, but prior to ligation (Post retraction-rCBF) in 24 randomly selected animals. The duration of anesthesia in all cases varied between 6–8 hours.

After surgery, the animals were allowed to awake and breathe spontaneously. All catheters were removed, the animals extubated, returned to individual cages and allowed to eat and drink ad lib. The animals were given Combiotic 1 ml i.m. and assigned daily neurologic scores according to the grading system of Crowell and Olsson:

- Grade I = No neurologic deficit.
- Grade II = Occasionally circles toward operated side. Stands up quickly without assistance. No field deficit to visual threat.
- Grade III = Circles toward operated side. Stands up only with assistance ± contralateral field deficit. No impairment of consciousness.
- Grade IV = Cannot stand ± contralateral field deficit ± drowsy.
- Grade V = Died.

One week after the initial surgery the animals were again anesthetized in exactly the same manner, catheters were replaced, the right hemisphere exposed, and rCBF repeated (1 week-rCBF). The animals were then given 6 ml of 25% sodium fluorescein i.v. and sacrificed 30 minutes later with 50 ml of intracarotid 10% formalin. The brains were carefully removed and clip placements verified. After 3 days of formalin fixation, percent infarction was determined by volumetric measurement of microscopically proven infarcted-fluorescent areas as described in detail elsewhere.

Non-parametric statistical tests of significance (Kruskal-Wallis Anova and Mann-Whitney U) were
TABLE 1  Regional Cortical Cerebral Blood Flows (ml/100 gr/min) ± SE

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Initial-rCBF</th>
<th>Postclip-rCBF</th>
<th>3 Hour-rCBF</th>
<th>1 Week-rCBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated-ligated</td>
<td>25</td>
<td>93.25 ± 4.70</td>
<td>48.11 ± 3.60</td>
<td>49.37 ± 4.06</td>
<td>45.15 ± 5.22</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>11</td>
<td>90.34 ± 6.07</td>
<td>—</td>
<td>73.60 ± 6.22</td>
<td>86.48 ± 1.36</td>
</tr>
</tbody>
</table>

TABLE 2  Neurologic Grade in the Untreated-Ligated Group Using the 7-Day Median Grade of Each Animal

<table>
<thead>
<tr>
<th>Neurologic grade</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>7</td>
<td>1</td>
<td>11</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Percent</td>
<td>28%</td>
<td>4%</td>
<td>44%</td>
<td>8%</td>
<td>16%</td>
</tr>
</tbody>
</table>

Cerebral Blood Flow

In the 24 randomly selected animals in which post-retraction rCBF was calculated, the initial-rCBF was 85.07 ± 3.93 and the post retraction-rCBF was 80.41 ± 5.18. This difference was not statistically significant (p > 0.05).

In the untreated-ligated group, initial-rCBF was 93.25 ± 4.70 and postclip-rCBF was 48.11 ± 3.60. This represents a 48.4% reduction in blood flow (p < 0.001). The 3 hour-rCBF was 49.37 ± 4.06 and the 1 week-rCBF was 45.15 ± 5.22, both of which were not significantly different from postclip-rCBF (p > 0.05) (table 1).

In the sham-operated group, Initial-rCBF was 90.34 ± 6.07, 3 hour-rCBF was 73.60 ± 6.22 and 1 week-rCBF 86.48 ± 10.36. This represents a transient 18.5% drop by 3 hours, (p < 0.05) with a return to initial blood flow levels by one week (table 1).

Neurologic Deficit

From the first day after surgery until the time of sacrifice, all sham-operated animals remained neurologically intact (Grade I), and there was no mortality.

The median neurologic grade of the 7 days for each animal in the untreated control group can be seen in table 2. In only 28% of animals the median grade was I (no neurologic deficit). Table 3 displays the number of animals (untreated-ligated) in each grade over the 7 days of the study. This data shows that there is a linear tendency for neurologic improvement as time goes on.

Mortality in this group was 16% (Grade V). One animal died on the first postoperative day, one on the second day, and 2 on the third postoperative day.

Cerebral Infarction

Evidence of fluorescent staining of the brain (infarction) was absent in all sham-operated animals.

In the untreated-controls some degree of infarction was seen in 20/25 (80%) of the animals. The mean percent infarction of the affected hemisphere was 17.00 ± 3.98. Infarction was confined to the distribution of the middle cerebral artery in all animals and was "bland" in all but 2 animals where there was evidence of gross hemorrhagic infarction, with one of these showing also intraventricular hemorrhage. Figure 2 is a graph of median neurologic grade vs percent infarction showing a good correlation between size of infarct and degree of neurologic impairment.
Discussion

The process of selecting an animal model for stroke research has been a difficult one. Anatomical anthropomorphism has been the principal criterion used for selection and, therefore, primates became the ideal candidates. Primates are now very costly and difficult to obtain. In addition, in the primates available for research, intracranial vascular structures are usually too small for microvascular surgery. The dog is a relatively inexpensive experimental animal which is easy to handle and readily available. It has proven to be a useful animal for intracranial microvascular surgery.

In spite of some theoretical disadvantages to a stroke model that requires an open cranium and dura mater, this is unavoidable if one is to perform intracranial vascular surgery research or the measurement of cortical cerebral blood flows using a beta emitter like $^{81}$Kr. In these situations, the additional surgery required to expose the circle of Willis unilaterally is negligible. Exposure of the internal carotid artery and its proximal branches requires some brain retraction but as our data show, this retraction has no effect on cortical blood flow and produces no neurologic deficit or infarction.

The selection of a 2 clip stroke method is based on our unpublished experience showing that this technique produces a greater and more consistent drop in cerebral blood flow and infarction than a single middle cerebral artery clip. In order to measure cerebral blood flows after an intracarotid bolus injection of radioisotope, the most proximal clip has to be placed distal to the posterior communicating artery, thus allowing the isotope to reach the middle cerebral artery distribution via posterior cerebral-middle cerebral collaterals.

This clip technique produced a mean drop in rCBF of 48.4% which persisted in time. In the sham-operated animals rCBF dropped 18.5% by 3 hours. This phenomenon has been previously described by several investigators.

The infarction frequency of 80% achieved with this technique is high, circumventing one of the major problems with dog stroke models. All the infarcts were limited to the vascular distribution of the middle cerebral artery and were non-hemorrhagic with only 2 exceptions.

Neurologic deficit was shown to have a positive correlation with percent hemisphere infarction as was expected.

References

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