Stability of Thrombosis Induced by Electrocoagulation of Rat Middle Cerebral Artery

Farouk El-Sabban, PhD; Kenneth H. Reid, PhD; Y. Ping Zhang, MD; Harvey L. Edmonds, Jr, PhD

Background and Purpose Although it is often assumed in experimental stroke studies that cautery-induced occlusion is permanent, surgeons commonly expect cauterized vessels to recanalize spontaneously. We used the rat middle cerebral artery to determine if electrocoagulation would produce a permanent occlusion in this preparation.

Methods and Results A standard bipolar coagulator, calibrated to determine actual power output, was adjusted to induce platelet aggregation in the middle cerebral artery of anesthetized Sprague-Dawley rats without inducing bleeding through the arterial wall. A reliable temporary thrombosis was induced by a Malis Bipolar Coagulator set to deliver 10 bursts of 1.5 seconds each at a rate of 24 min⁻¹ and a power setting of 3 W. This thrombus was responsive to the antithrombotic agent flunarizine. An apparently permanent occlusion was produced by 30 bursts at 3 W followed by 20 bursts at 5 W. To our surprise, seven of seven such occlusions recanalized spontaneously within 4 hours.

Conclusions The electrocoagulation process commonly used in experimental stroke studies may produce only a temporary occlusion of the rat middle cerebral artery. (Stroke. 1994;35: 2241-2245.)

Key Words • cerebral circulation • cerebral ischemia, transient • electrocoagulation • platelet aggregation • rats

Oclusion of the middle cerebral artery (MCA) in the rat is a common in vivo model for local cerebral infarction. Bipolar cautery is commonly used to perform the occlusion. The permanence of such an occlusion has not been established, although several researchers have assumed that cautery-induced occlusion is permanent. In surgical practice, blood vessels are cauterized to control bleeding, but the occlusion is assumed to be temporary; the surgeon expects the thrombus that is formed to embolize and flow to be restored. This assumption contradicts the assumption made in the infarct models cited above.

The purpose of the present study was to reliably produce controlled platelet aggregation using a bipolar coagulator for use in an animal model of stroke. Because we could find no relevant data in the literature, we evaluated the power output of our coagulator using a simple physical model. We studied the time course of thrombotic and embolic events in the MCA of the rat with the aim of defining parameters of electrocoagulation sufficient to establish either a stable temporary thrombus or a permanent occlusion of blood flow. The stable thrombus preparation was used to test the efficacy of a known antithrombotic agent, flunarizine.

Materials and Methods

Male Sprague-Dawley rats (weight, 200 to 350 g) were kept in a temperature-controlled room with a regulated 12-hour light-dark cycle. Food and water were provided ad libitum. All experiments were conducted during the midportion of the light period. All procedures followed institutional guidelines.

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Rats were anesthetized with sodium pentobarbital (50 mg/kg IP), with additional supplementation as needed. A tracheotomy was routinely done to maintain an open airway, and rectal temperature was maintained at 37°C with a heating pad. Exposure of the MCA was carried out according to the method of Tamura et al. Briefly, the rat was placed on its side, and the head was restrained with a stereotaxic headholder. A 12-mm skin incision was made between the lateral corner of the left eye and the external auditory orifice. The platysma was separated, and the anterior superficial masseter, posterior superficial masseter, and temporalis muscles were divided and partially removed. The posterior part of the zygomatic bone was exposed and divided. The deep masseter muscle was retracted, and the mandible was pulled downward to expose the base of the skull. The bone 3 mm lateral and 1 mm anterior to the foramen ovale was thinned down with a dental drill cooled with cold saline and then removed. The underlying dura was opened to expose the MCA (Fig 1).

The electrode used for electrocoagulation was made of two tungsten rods (0.013 mm in diameter, A-M Systems, Inc) mounted in theta glass tubing. The tip of each electrode was tapered by electrolysis in a sodium nitrite solution to a tip diameter of 20 to 25 μm. Each rod was insulated with a Teflon coating so that a length of 80 to 120 μm of the tip was exposed. The two tips were 0.2 mm apart.

The radiofrequency generator used was a Malis Bipolar Coagulator (model NS-237, Codman and Shurtleff, Inc). The power output could be set from 10 to 90 arbitrary units with a front panel switch. To control the duration of radiofrequency application, a timing relay circuit was added so that each application of radiofrequency via the foot switch lasted precisely 1.5 seconds.

The power settings of the coagulator were converted to watts by passing the current through a 5-W 120-Ω resistor with a No. 30 wire thermocouple glued to its surface to measure the temperature rise produced. This procedure was necessary, as this spark-gap-type coagulator produced a complex and irregular waveform that was too unstable for the calculation of power from oscilloscope measurements of voltage and current. The temperature rise produced by current from the coagulator was compared with the rise produced by a known direct
The power associated with that current was calculated using the following relation:

\[ \text{Power} = (\text{Current})^2 \times \text{Resistance} \]

The radiofrequency component of the coagulator output was found to interfere with the circuitry of a Sensortek Model BAT 12 thermistor thermometer; thus, temperatures were read immediately after the current was turned off. Cooling curves were recorded and used to extrapolate to zero time to verify the measured temperature readings. From the cooling curves (Fig. 2A), the time constant of the system was determined to be 1.4 minutes. The equilibrium temperature rise was calculated from the rise at known times using the following relation:

\[ T(t) - T(0) = [T(\text{equil}) - T(0)] \times [1 - \exp(-t/\tau)] \]

where \( T(t) \) is the temperature at time \( t \) (minutes), \( T(0) \) is the temperature before applying power, and \( T(\text{equil}) \) is the temperature after a time much longer than \( \tau \), the system time constant. The temperature rise \( T(\text{equil}) - T(0) \) is plotted against known power (direct current) in Fig. 2B. Temperature rise measurements were used to determine the power output of the coagulator at various settings. These results are plotted in Fig. 2C.

A three-axis micromanipulator was used to place the coagulating electrode accurately on the MCA. The MCA preparation was kept wet with saline during the coagulation procedure. To determine a power setting that would reliably produce platelet aggregation without inducing hemorrhage due to damage to the arterial wall, a series of tests were run at different settings. Each setting was tested on the proximal or distal parts of the MCA (Table). Formed thrombi, as seen on the television monitor, appeared as white structures resembling platelet aggregates attached to the lumen of the arterial wall. Based on these data, a standard dose for thrombus formation was selected to be power setting of 25 (3 W); burst length, 1.5 seconds; interburst interval, 1.0 second; and total number of bursts, 10.

In an additional seven rats, we used more prolonged and intense electrocoagulation with the aim of permanently occluding the MCA: 30 bursts at setting 25 (3 W) followed by 20 bursts at setting 30 (5 W). The MCA was monitored with television microscopy for as long as 4 hours to determine the duration of the occlusion produced.

Five groups of rats (seven per group) were used in a trial of the antithrombotic agent flunarizine. One group was injected with saline (control); another group was injected with cyclodextrine, the solvent of flunarizine (vehicle); and the remaining groups received flunarizine in cyclodextrine. Graded doses of 0.1, 0.4, and 1.25 mg/kg flunarizine were tested. All injections were made via the tail vein 15 to 20 minutes before coagulation. These doses were based on clinically effective doses used in previous studies.

All coagulations were proximal to the largest branch of the MCA. The time and number of thrombi formed and number of emboli seen were recorded manually. The observation time chosen, 1 hour, was based on preliminary observations for as long as 4 hours. The field of observation was videotaped with a television microscopy system for later mea-
Results of Coagulation of Proximal and Distal Sites of Middle Cerebral Artery at Different Coagulator Power and Burst Settings

<table>
<thead>
<tr>
<th>Coagulator Power Setting</th>
<th>No. of 1.5-s Bursts</th>
<th>Middle Cerebral Artery Site</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Proximal</td>
<td>Thrombosis No</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Hemorrhage No</td>
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<tr>
<td>2</td>
<td>20</td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td>Distal</td>
<td>Thrombosis No</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Hemorrhage No</td>
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<tr>
<td>3</td>
<td>25</td>
<td>10</td>
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<tr>
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<td></td>
<td>Proximal</td>
<td>Thrombosis Yes</td>
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<td></td>
<td></td>
<td>Hemorrhage No</td>
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<td>30</td>
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<td></td>
<td>Hemorrhage No</td>
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<td>Thrombosis No</td>
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<td></td>
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<td></td>
<td>Hemorrhage Yes</td>
</tr>
</tbody>
</table>

These results were used to select the power levels to be used for temporary and "permanent" electrocoagulation of the middle cerebral artery (see "Materials and Methods").

The MCA diameter was measured 10 minutes before coagulation, immediately before coagulation, and at 5, 15, 30, and 60 minutes after coagulation. All diameter measurements were made to the nearest millimeter on the 21-in monitor of the microscope-closed-circuit television and were converted to true millimeters using a known magnification factor.

We used a one-way analysis of variance to detect differences among the five groups of this trial. This was followed by a Dunnett's multiple comparison of the control against the vehicle and dose groups.

Results

The standard coagulation procedure induced MCA thrombosis without hemorrhage that lasted for at least 60 minutes—time long enough to study the effect of the antithrombotic agent flunarizine. Formed thrombi grew in size and then disintegrated into emboli. Emboli were counted either when the whole thrombus became loose or when countable pieces broke off. This standard coagulation dose did not cause the vessel to occlude permanently.

When cauterization was continued until the MCA was totally occluded (seven rats), all vessels were recanalized within 4 hours (160±73 minutes). None of the recanalizations was complete, as some thrombosis always persisted after blood flow was resumed. We were unable to follow these preparations for a long enough time to determine whether these residual thrombi would eventually clear.

In the flunarizine trial, the average time from the end of the coagulation to development of the first thrombus in the MCA of the five groups is shown in Fig 3. The group receiving 1.25 mg/kg flunarizine had a significantly (P<.05) prolonged time to the appearance of the first thrombus compared with the control group (4.6 ±1.0 versus 2.5 ±0.6 minutes, n=7). Lower doses of flunarizine or the vehicle alone had nonsignificant effects.

The duration of thrombotic activity in the MCA of the five groups of the flunarizine trial is shown in Fig 4. Thrombi resolved spontaneously in both treated and control preparations. Although thrombus clearance times did not differ significantly between groups, in two of the seven rats receiving 1.25 mg/kg flunarizine, it was impossible to produce a thrombus using the standard level of electrocoagulation.

The numbers of emboli and thrombi observed in the five groups are presented in Fig 5. Emboli were significantly (P<.01) reduced by the 0.4 and 1.25 mg/kg flunarizine doses compared with the control group. Thrombi were significantly (P<.05) reduced by the 1.25 mg/kg dose only.

Electrocoagulation produced arterial dilation in all groups, which persisted throughout the 60-minute observation period. This did not differ significantly among the groups. The overall change from original diameter was 64.4±5.6%.

Discussion

Bipolar cautery can be used to reliably produce thrombosis without hemorrhage in the rat MCA, provided that the radiofrequency energy applied is held...
Fig 4. Bar graph of duration of thrombosis for the five groups shown in Fig 3. Time was measured from the formation of first thrombus until the time at which no more thrombi were attached to the artery wall, during a 1-hour observation period from beginning of coagulation. Groups are labeled as in Fig 3. n = 7 for all groups except 1.25 f, where thrombosis could not be produced in two of seven rats (see "Results"). Bars are 95% confidence limits.

within strict limits. With the Malis coagulator, the optimum power level for producing a temporary thrombus was approximately 3 W if coagulation was applied in 1.5-second bursts at a rate of 24 min⁻¹. This electrocoagulation was found to last long enough (60 minutes) to test the protective effect of a drug (flunarizine) against vascular trauma. The drug was found to have a significant effect on number of emboli, number of thrombi, and time to first thrombus. These effects are consistent with the known cerebroprotective properties of flunarizine.15,16,18,19

We were unable to locate a detailed biophysical description of the radiofrequency electrocoagulation process we used for occlusion of the rat MCA. The electrocoagulation of nervous tissue has been analyzed by neurosurgeons.20,21 Temperature was found to be the best measure of tissue damage; the electrode tip used for neurosurgical procedures contains a thermistor thermometer. The smallest available tip of this type was 0.25 x 2 mm (Radionics)21; we did not have access to such an instrument. In cardiac surgery, electrocoagulation has been used to treat drug-refractory ventricular tachycardia; voltage, current, and phase angle measurements were found to be useful in determining lesion size and depth,22 and multiple brief applications of radiofrequency energy produced more reliable lesions than a single prolonged application, a result consistent with our experience with the rat MCA.

Direct current was used for arteriole coagulation in early studies.23,24 From 1 to 2 mA applied for 5 minutes over a 4-mm length produced thrombosis in rat carotid arteries that lasted for at least 15 minutes. The thrombi consisted of platelets, with some red blood cells.24 Histological evaluation indicated that the endothelium was severely damaged by this procedure.23 Lasers have also been used, both experimentally21 and clinically20,23-26; the critical parameter, as in the neurosurgical studies, was found to be temperature. For a 20- to 30-μm-diameter mesenteric vessel, pulses of 120 mW x 67 milliseconds were effective; one such pulse produced a small thrombus, and three to five repetitions at 1-minute intervals produced a thrombus that half-filled the vessel.25

In our study, when electrocoagulation was used to produce a "permanent" block of blood flow in the MCA, it did not do so. With a maximal nonhemorrhagic electrocoagulation, we observed only a temporary occlusion, which lasted for less than 4 hours. On the basis of our data, the assumption that bipolar coagulation produces a reliable permanent occlusion of the rat MCA11,12,17 is questionable. Stroke models using this form of occlusion may better represent "temporary ischemic attacks" than permanent occlusion.

Does recanalization matter? In studies where infarcts are produced and verified, it would seem not. One hour, or even half an hour, of no blood flow will produce an infarct. In the study by Lye et al.,11 infarcts of widely varying size were reported; the variation may have been due in part to variations in the duration of the MCA occlusion. The study by Sauter and Rudin12 reported much less variability, and drug treatment that halved infarct size was statistically significant. Neither study provided any details on the technique of coagulation.

In studies where the evidence sought is the lack of an infarct, recanalization may be more important. Rubino and Young17 coagulated branches of the MCA and used Evans blue, injected before or shortly after cauterization, to detect damage to the blood-brain barrier. Coagulation of frontal or parietal branches of the MCA did not produce lesions in approximately 40% of the rats. These results were used to infer substantial collateral circulation in the MCA territory. While collateral circulation certainly exists, recanalization could also contribute to a lack of lesions in some of the animals studied. The combination of coagulation and cutting used by other researchers2,3,6,8,27 provides a stronger assurance of permanent interruption of blood flow in the rat MCA preparation.
References


Editorial Comment

The preceding article by Dr El-Sabban and coworkers establishes another caution for those using animal models of cerebrovascular occlusion. The authors show that a technique that has been assumed by others to produce permanent occlusion does not do so. Instead, the electrocautery used produced temporary occlusion only, with embolization and partial recanalization following. If this model were used to interpret the basis of either infarction or therapeutic efficacy of drug treatment, the following questions would be raised. Were the infarcts embolic rather than thrombotic in origin? Was therapy efficacious because of an effect on thrombosis or because of an effect on the frequency or fate of emboli? Was failure of therapy related to use of an intervention whose effect is directed toward thrombosis, in a model where thrombosis is only part of the etiology of infarction? The authors’ work raises an important point of concern. Their data are restricted to the rat. One wonders whether a similar concern should be raised if the technique is used in another species and/or with their data are restricted to the rat. One wonders whether a similar concern should be raised if the technique is used in another species and/or with different parameters of current intensity or frequency.

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