A model of focal cortical contusion in gerbils

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An experimental model of focal laceration and contusion in gerbils is described. Associated with this injury are systemic changes which are neurogenically mediated and result in an immediate reduction in blood pressure, bradycardia, and generalized reduction in cerebral blood flow. There is generalized edema, as judged by a decreased specific gravity in the brain, probably related to reduced blood flow; superimposed on this, there is an edema gradient which is maximal close to the injury. This, in turn, affects the local capillary bed and prevents any local increase in flow. A separate group studied over a longer time period (6 hours) did not reveal egress of Evans blue into the surrounding tissue and this is in contrast to reports from cold-injury studies.

KEY WORDS - brain injury - brain edema - cerebral blood flow - experimental model

A wide variety of experimental models have been designed to evaluate specific aspects of cerebral trauma. The baboon extradural injury model has emphasized the effects of brain shifts and reduction in cerebral perfusion pressure. Models of focal and global ischemia have provided useful information on the limits of tolerance of neural tissue to low blood flow and identical values have been obtained following missile injury. At low flows, there are changes in extracellular potassium, and similar findings have been reported in experimental concussion. Various methods of inducing brain edema have been studied, and the cold-injury model has been used extensively to study the pathophysiology of vasogenic edema. This phenomenon occurs around a tumor, and there has been a belief, unsubstantiated by data, that the cold-injury type of edema is responsible for swelling after closed-head injury: it has been used as the model to evaluate therapy for trauma.

The effects of injury are more complex than the effects of extreme cold, however, and may include hypoxia, ischemia, and lacerations of blood vessels and brain tissue, which would release vasoactive substances and these in turn could produce secondary effects. The purpose of this research was to establish, if possible, a small animal model which allowed study of flow, edema, and the release of vasoactive substances from an area of cerebral contusion and laceration.

Materials and Methods

Preparation

Adult male gerbils, weighing 40 to 60 gm, were used in this study. The animals were anesthetized with pentobarbital, 60 mg/kg body weight, injected intraperitoneally, and allowed to breathe spontaneously. Barbiturate supplements, 15 mg/kg, were given as required. No animal was allowed to recover from the anesthetic, which was maintained until sacrifice with barbiturate overdose. A No. 32 catheter was inserted into the femoral artery for measurement of blood pressure, heart rate, and blood gases. A sagittal skin incision allowed reflection of the scalp. Four burr holes were carefully drilled, two bifrontal and two biparietal, in positions identical to those in our stroke model. Four platinum-iridium Teflon-coated electrodes, 1 mm long and 100 µm in diameter, were inserted into the gray matter through the burr holes and held in position with methyl methacrylate. A silver-silver chloride reference electrode was inserted subcutaneously in the dorsal region.

The right eyeball was removed, and any minor bleeding points controlled with bipolar coagulation. The animal was then placed in a stereotaxic head frame. An electric drill with a 2-mm drill bit was clamped in a side arm attached to the stereotaxic head frame, and the cortical injury was produced by moving the carefully positioned drill along a predeter-
FIG. 1. Diagram showing the method of producing the focal cerebral contusion and laceration in this gerbil model. Blood flow (rCBF) was measured in four areas and correlated with specific gravity (S.G.) measurements in the adjacent brain. White matter from two other regions was assessed also for water content. The stereotaxic method allowed reproducible injuries.

FIG. 2. Coronal sections of formalin-fixed gerbil brain to show the gross appearance of the severe injury.

A. Crockard, J. Kang and G. Ladds

mined path extending from the right orbit backward and medially up to the midline, a distance of 4 mm (Fig. 1). In the development of the model, the degree of injury was adjusted to provide a "severe injury" which had a 30% mortality within 2 hours of injury, and a "mild injury" which resulted in no deaths over the first 2 hours. The brains from these animals were fixed in formalin and sectioned to confirm the site and extent of the contusion and laceration (Fig. 2). A mild injury was produced by moving the drill along this pathway at 50 rpm over 2 seconds, and a severe injury was produced in a similar fashion at 200 rpm for 2 seconds. After the injury, the orbit was sealed with bone wax. The direction of the injury was chosen to avoid major cerebral vessels. Bleeding from the orbit was minimal. Two groups of 20 animals were used to compare the effects of the differing degree of injury.

In a separate series, 12 animals were injected intravenously with 2% Evans blue buffered solution (1 mg/kg body weight) and injured in an identical fashion. They were then sacrificed, perfused with saline, at 1, 2, 4, and 6 hours after injury, and their brains sectioned to estimate the spread of the dye.

**Experimental Procedure**

The animals' temperature and vital signs were carefully kept stable; we discarded any experiment in which there was a departure from normal values. Control observations of blood pressure, heart rate, blood gases, and cerebral blood flow (CBF) were carried out. Immediately after injury, the observations were repeated, and further blood flow estimations were performed at 30 minutes and before sacrifice, 1 hour after injury. The animals were sacrificed at 1 hour and the brain rapidly removed, inserted into a bromobenzene-kerosene solution, and dissected under the operating microscope to assay the specific gravity of the right and left frontal region, right and left parietal region, and right and left white matter, that on the right being adjacent to the area of damage.

**Measurements**

All measurements were standard for our gerbil preparation and are described in detail elsewhere. Blood pressure and heart rate were monitored via a femoral artery catheter connected to a Statham P23Gb transducer. Blood gases were assayed on a Radiometer ABL3 blood gas analyzer using 0.2-ml aliquots of blood. No more than three samples were taken from any animal. Focal cerebral blood flow (rCBF) measurements were obtained using the hydrogen clearance technique, and the calculations made by the initial-slope technique.

Specific gravity of the brain was measured as described by Nelson, et al., with the modifications of Marmarou, et al., using the bromobenzene-kerosene column gradient. Four to six samples of brain, 1 cu mm in size, were carefully dissected with the operating microscope from the tissue surrounding each elec-

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* Statham P23Gb transducer manufactured by Statham Instruments, Inc., 2230 Statham Boulevard, Oxnard, California.
† Radiometer ABL3 blood gas analyzer manufactured by Radiometer A/S, 72 Emdrupvej, DK2400, Copenhagen, Denmark.
Experimental model of focal cortical contusion

Fig. 3. A slow (upper) and fast (lower) record of the changes in blood pressure (BP) following severe injury. After a transient rise, blood pressure falls and remains low following injury. Note the transient changes in pulse rate, pulse pressure, and pulse irregularities, occurring immediately after injury (fast record); these changes are considered to be neurogenically mediated.

trode; their specific gravity was assayed, and the result expressed as an average of the four to six samples.

Results

Systemic Changes

In the control period, the mean blood pressure for the mild and severe groups was 78 ± 8 mm Hg with a heart rate of 142 ± 10 beats/min (Table 1). The arterial blood gases in this period were PaO2 86 mm Hg and PaCO2 35 mm Hg. During or immediately after the injury, there was bradycardia, an increased pulse pressure, and occasionally a transient rise in blood pressure, but more usually a fall in blood pressure to about 50 mm Hg. Occasionally, there were cardiac irregularities, which were transient. The blood pressure never returned to control values. The changes were most marked in the severely injured group and, although they were present in animals with a mild injury, the changes were less dramatic (Fig. 3). Immediately after injury, a transient apnea was observed for a few seconds. This was followed by an increase in respiratory rate and rhythm for the rest of the period of observation.

Regional Cerebral Blood Flow

Control blood flow results from each group were obtained by averaging the four regional results from each animal and taking a mean of this result for the group. The mean control rCBF for the mild injury group was 31.1 ± 1.5 ml/100 gm/min. The control observations in the severe injury group were similar, at 36.3 ± 3.8 ml/100 gm/min. Immediately following injury in both the mild and the severe injury groups, there was a marked reduction in blood flow in all regions to values between one-third and one-half of the original control values (Fig. 4). The blood flow values were identical for the severe and the mildly injured groups, ranging between 10 and 16 ml/100 gm/min. At 30 minutes and 1 hour after injury the regional flows varied.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Severe Injury</th>
<th>Mild Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart Rate</td>
<td>Mean Blood Pressure</td>
</tr>
<tr>
<td>control</td>
<td>142 ± 10</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>at 1 minute</td>
<td>154 ± 23</td>
<td>43.3 ± 16</td>
</tr>
<tr>
<td>at 30 minutes</td>
<td>162 ± 12</td>
<td>61 ± 12</td>
</tr>
<tr>
<td>at 1 hour</td>
<td>157 ± 19</td>
<td>56.2 ± 10</td>
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</tbody>
</table>

* Mean heart rate (beats/min) and mean blood pressure (mm Hg) values ± standard error.

In general, it can be said that in the mild injury group, blood flows did not change from values obtained immediately after injury. The results from the group as a whole were homogeneous, as demonstrated by the small standard errors on the group results at 30 and 60 minutes. The results from the severe injury groups showed much more variation, but there was generally an increase in flow at 30 minutes in all areas except the right parietal region. In five animals, rCBF reached control values in these areas. At 1 hour, rCBF in the same regions was slightly reduced. Blood flow in the right parietal area (directly above the injury site) remained low following injury in both the mildly and severely injured groups.

Brain Specific Gravity

Normal brain specific gravity measurements obtained from animals subjected to surgical preparation but no injury averaged 1.0495 ± 0.0003; this value was similar for frontal and parietal regions and also for white matter. Following injury, there was a significant decrease in the brain specific gravity of all regions in both the mildly and severely injured groups with the maximal edema in the right parietal region (above the injury site), followed by the right frontal region. There was significantly less edema in the left

J. Neurosurg. / Volume 57 / August, 1982

205
FIG. 4. The grouped mean cerebral blood flows (CBF) from four regions after cerebral laceration. Following injury, there is a global reduction in flow. In the severely injured animals (black circle), flow increased in all areas except those adjacent to the injury site. Flow following mild injury (open circle) remains low in all areas following injury consistent with the lower blood pressure. RF = right frontal; RP = right parietal; LF = left frontal; and LP = left parietal regions.

hemisphere, the least being detected in the left frontal region. The white matter on the left hemisphere was not significantly different from control values. Comparing both groups, there was significantly more edema in the severely injured group in each region, apart from the white matter close to the injury site (Fig. 5).

Relationship of Flow to Edema

As in our previous work with ischemia, we have attempted to correlate rCBF values with brain specific gravity measurements. Unlike the ischemia model, we have found no obvious correlation between the focal flow and the focal brain specific gravity measurements. Neither the lowest flow obtained in the region nor the flows at 30 minutes or 1 hour produced any obvious correlation (Fig. 6).

Evans Blue Staining

There was no spread of dye into the surrounding tissue beyond the contused area, even 6 hours after injury.

Discussion

Cold injury has been used in a variety of animal species as a model of vasogenic edema, and there has been a tendency to use the results obtained to draw inferences relating to clinical brain trauma. While this may be so, it is difficult to find evidence to link the cold-injury model with the clinical situation presented by acute head injury. With increasing use of computerized tomography, there is a growing awareness that the intracranial effects of trauma may include a combination of contusion and intracerebral hematoma, graphically described as a “burst lobe.” In this model, therefore, we have attempted to produce a significant focal contusion, a laceration of brain and vascular tissues with extravasation of blood into the contusion area in an attempt to mimic this aspect of the clinical condition. While producing a significant local lesion, there have been marked systemic effects also, which, because of their immediate appearance after injury, can only be neurogenic. Similar changes of hypotension, bradycardia, and respiratory variations have been described with the
Experimental model of focal cortical contusion

Fig. 5. The specific gravity (S.G.) of brain for control (C), severely (S), and mildly (M) injured animals is shown for gray and white matter from the regions described. Specific gravity in control or sham animals was 1.0495 to 1.0489, and following injury there was a significant decrease in S.G., in all areas except the left frontal area, which was furthest away from the injury. The gray and white matter close to the injury had most edema.

These systemic cardiovascular effects are probably responsible for the generalized reduction in flow in all regions immediately after injury. This would be in keeping with the findings of Levett, et al., who showed a reduction in cardiac output commensurate with the decrease in CBF in experimental missile injury. These cardiovascular effects were described by Fozzard in experimental burns 20 years ago, and also reported in experimental thigh injury in the dog. There are similar findings in clinical and experimental subarachnoid hemorrhage.

Persisting hypotension following injury in our model would account partly for the reduction in CBF. The global CBF reduction with focal injury may be explained by a "central" disturbance of autoregulation, due to the generalized effects of trauma or because the site of injury extended to midline structures. This global effect was also noted in missile injury, but there it was believed that explosive energy was transmitted to all the intracranial contents; in this model we consider the trauma to be severe but focal. The levels of flow that we measured (10 to 16 ml/100 gm/min) have been associated with the production of edema in our gerbil ischemia model. The generalized ischemia immediately after injury may cause the local accumulation of metabolites, to account for the slight increase in flow in three of the regions 30 minutes and 1 hour after injury. The lack of any increase in flow in the right parietal region, which was directly above the site of injury, may have been due to local factors. It was the area with most water, and the local tissue pressure may have prevented the vasodilatory effects of the metabolites.

A reduction in flow to less than 16 to 20 ml/100 gm/min has been associated with an accumulation of water in the gerbil and this would account for the increase in water in areas most remote from the injury. There may be a severe reduction in flow at the moment of injury and the persisting low flow would produce a degree of ischemia with reperfusion, pro-
Figs. 6. The lowest recorded regional cerebral blood flow (rCBF) at each electrode was plotted against the final specific gravity (S.G.), 1 hour after injury. There is no obvious correlation between flow and edema in this severe injury model. RF = right frontal; RP = right parietal; LF = left frontal; and LP = left parietal regions.

Producing "reperfusion" edema. In ischemia, we have been able to correlate flow with the amount of water accumulation; we have also shown that there is a relationship between remaining flow during ischemia and the resolution or aggravation of edema. However, we could not show any relationship in the trauma model between flow and edema, and this is due to the complexity of events following trauma. There are clearly factors related to the injury itself in that there is most edema in areas closest to the injury site.

This is in keeping with the findings of Tornheim and McLaurin, investigating a temporal contusion in the cat. We were unable to show the marked increase in white matter edema that others have noted following vasogenic edema for trauma. Part of the problem may be the difficulty in isolating white matter edema in such a small brain as that of the gerbil, but it may also be the result of the time factor in that our model had an end point 1 hour after trauma, whereas others who have reported the spread of white matter edema have usually used a longer time scale (24 to 48 hours).

In the separate series in which Evans blue was administered there was no obvious staining of surrounding tissues 6 hours after injury and this has been our experience (unpublished) in the missile model. In cold injury, there is very obvious staining at this time, and thus we question if the cold-injury model reflects the pathophysiology of a cerebral contusion and laceration.

References

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Experimental model of focal cortical contusion


Manuscript received January 11, 1982.
This paper was supported by grants from The National Hospital. Dr. Kang was supported by the Catholic University, Seoul, South Korea.
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