Effects of experimental intracerebral hemorrhage on blood flow, capillary permeability, and histochemistry


Department of Neurosurgery, University of Glasgow, Institute of Neurological Sciences, Southern General Hospital, Glasgow, Scotland

Experimental intracerebral hemorrhage has been shown to cause extensive cerebral ischemia. This study was performed to ascertain the time course of these changes and also to examine the type of brain damage that may occur under such circumstances. Halothane anesthesia was induced in rats, and 25 µl autologous blood was injected into the caudate nucleus; the effects were studied with autoradiographic measurement of local cerebral blood flow and capillary permeability, and also by light microscopy and histochemical techniques. Blood flow returned to normal or to slightly increased levels within the first 3 hours, and ischemic levels of flow were found to persist only to a marginal degree beyond 10 minutes after the lesions were made. Capillary permeability was maximum during the first 30 minutes after the hemorrhage and diminished with time. Structural evidence of ischemic damage was localized to the cortex overlying the hemorrhage, but was not seen in the caudate nucleus. Nevertheless, histochemical investigation did reveal an area of disturbed enzyme function in the striatum. This finding of biochemical disturbance without structural evidence of ischemic damage reveals that there is an area around the hematoma that, although demonstrating disturbed function, does not show structural damage, and the milieu of this partially injured brain may be implicated in the delayed development of the ischemic brain damage that follows intracerebral hemorrhage in man.

KEY WORDS • intracerebral hemorrhage • blood flow • ischemia • capillary permeability • histochemistry • rat

DELAYED deterioration may follow initial survival from cerebral hemorrhage due to either trauma, arterial aneurysm rupture, or hypertension. Explanations for delayed events include late changes in cerebral blood flow (CBF) secondary to vasospasm, disturbed cerebral metabolism, and/or altered blood-brain barrier (BBB) function. It is also possible that brain damage caused by the hemorrhage is only revealed in response to an added systemic insult, such as hypoxia, hypotension, or hypoglycemia brought about by dehydration, cardiorespiratory changes, or surgery. Previous studies using an experimental model have shown that, at the moment when a bleed occurs, there is significant cerebral ischemia. The first purpose of this study was to plot the evolution of this ischemia by measuring CBF at different times after hemorrhage. The second purpose was to explore over time the effect of the bleed upon BBB permeability and upon neuronal structure and function.

A rat model of arterial hemorrhage was studied by quantitative autoradiography to measure blood flow and cerebrovascular permeability. Standard neuropathological techniques were used to assess ischemic neuronal damage, and enzyme histochemistry was employed to detect disordered enzyme activity.

Materials and Methods

Adult male Sprague-Dawley rats, each weighing 350 to 400 gm, were fasted for 12 hours then anesthetized with halothane in a 70:30 mixture of nitrous oxide and oxygen delivered through a tracheostomy. Bilateral femoral arterial and venous lines were introduced, and the arterial blood pressure was monitored throughout the experimental period. Each animal was maintained under normoxia (PaO₂ > 100 mm Hg) and normocapnia (PaCO₂ 35 to 40 mm Hg) using intermittent positive-pressure ventilation. Body temperature was maintained at 37°C by an external heat source, and the blood glucose level was measured within 10 minutes of hemorrhage production.
Intracranial pressure (ICP) was measured by stereotaxic insertion of a fine (No. 18) polythene catheter into the body of the left lateral ventricle through a 1-mm burr hole. This catheter was held in place with zinc dental cement. An obvious arterial pulsation and a rise in pressure on gentle abdominal squeezing confirmed ventricular placement, but no intracranial blood injection was made.

Production of Hemorrhage

In this investigation, 25-μl lesions were studied. These were produced by injecting fresh unheparinized femoral arterial blood through a No. 25 needle connected to a tube graduated in 25-μl steps. A mercury column connected to a saline reservoir via an underwater seal was used to generate an injection pressure of 100 mm Hg. After 0.5 ml arterial blood was withdrawn, an equal volume of saline replacement was given through the femoral vein. The graduated tube and needle were then filled with the blood and were connected to the saline column by a three-way tap. A small air bubble served to separate the blood from the saline and to indicate the volume of blood injected by its movement along the graduations in the tube. The needle was then placed stereotaxically into the caudate nucleus, and 25 μl blood was injected intracranially via the three-way tap. The injection was terminated by closing the tap. The entire procedure was always completed within 24 minutes of withdrawal of blood, and the needle was kept in place for 45 minutes, or as appropriate to the experiment.

Control Studies

Sham-operated animals served as controls. These were prepared in an identical fashion to the lesioned animals including withdrawal of blood and saline replacement, but no intracranial blood injection was made.

Blood Flow Measurements

Blood flow was measured at three time intervals after the hemorrhage: 1 minute, 10 minutes, and 3 hours after lesion production. Each time study involved five animals with lesions and five sham-operated control animals. At the selected time after hemorrhage a ramped infusion of carbon-14 (14C)-labeled iodoantipyrine was injected through a venous line using an infusion pump, as described by Sakurada, et al. A 50-μCi dose isotope in 1.5 ml saline was administered over a 30-second period, during which freely flowing femoral arterial blood was sampled at known time intervals on preweighed filter-paper discs. Each rat was sacrificed immediately after isotope infusion by means of a fast intravenous bolus dose of 14C-aminoisobutyric acid (AIB).

Evans Blue Dye Infusion. In five animals a lesion was produced and, after an interval of 1 hour, 1 ml of 2% Evans blue dye in saline was filtered and injected intravenously. After a further hour the brain was removed, immersed in a solution of 40% formaldehyd, glacial acetic acid, and absolute methanol for 4 days, and sectioned. The distribution of Evans blue dye was drawn on the same charts that were used to map the distribution of ischemic damage. Five sham-operated animals served as controls. In a further three animals, the caudate nucleus was examined by fluorescence microscopy after cryosection of the brain. This confirmed that the discoloration observed was indeed Evans blue dye.

Autoradiography. Six animals were studied with quantitative autoradiographic techniques immediately after lesioning and five at 24 hours after lesioning. There were two groups of time-matched controls, with five animals in each group. Fourteen arterial blood samples were obtained at known time intervals over 25 minutes after injection of AIB. The blood was centrifuged and a fixed volume withdrawn for liquid scintillation counting. Immediately after the last blood sample had been taken, the brain was rapidly removed and processed in exactly the same way as in the blood flow experiments except that the samples were exposed to the x-ray film for 3 weeks. Based on the same microdensitometric techniques for measurement of tissue 14C concentration as for the blood flow measurements, and also the known blood history of the isotope, the intensity of the cerebrovascular permeability was determined from the formula derived by Ohno, et al.\(^6\)

\[
K_i = \frac{C_t (T)}{C_0 T} = \frac{\text{Concentration of tracer}}{\text{Exposure to tracer}},
\]

where \(K_i\) is the blood-to-brain transfer constant for the

\(\text{Capillary Permeability}\)

Capillary permeability was assessed in two ways. The first method consisted of measuring the distribution of Evans blue dye extravasation. The second method involved the quantitative autoradiographic technique developed by Blasberg and his colleagues, utilizing an intravenous bolus dose of 14C-aminoisobutyric acid (AIB).

The filter-paper discs containing the arterial blood samples were reweighed, and the volume of blood on each disc was calculated. The isotope concentration in each blood sample was measured by liquid scintillation counting after the blood was allowed to elute into the counting medium for 24 hours. The cerebral isotope concentration was measured by means of a computer-based microdensitometric method. The local CBF was calculated from the known blood history and the blood-to-brain partition coefficient for one isotope concentration using the formula originally derived by Kety.\(^6\)

F. P. Nath, et al.
Effects of experimental intracerebral hemorrhage

isotope, \( T \) the experimental time, \( C_b \) the concentration of tracer in the brain, and \( C_p \) the concentration of tracer in the plasma. In a normal brain, the \(^{14}\)C-AIB isotope (a neutral and metabolically inert low-molecular-weight amino acid) is transported slowly across the capillary endothelium but is rapidly carried from the extracellular space across cell membranes where it becomes trapped. The \( K_i \) factor is a measure of the rate of transport of the amino acid into the cell.

The autoradiograms were also examined to assess lesion size. Each section was cut in the same way with a 200-\( \mu \)m interval between each. Thus, the number of sections showing the presence of radioactive isotope was a measure of size. The product of intensity (\( K_i \)) and the number of sections showing increased uptake were used to compare lesion size with the severity of the changes.

Neuropathology

After the experimental hematoma was produced, the animals were maintained lightly anesthetized and the experiment was terminated after 3 to 4 hours by intracardiac perfusion fixation with a formaldehyde/methanol/acetic acid mixture, according to the method of Brown and Brierley. The brains were removed after a further 12 hours of immersion in fixative, the cerebral hemispheres were sectioned into four coronal slices, and the hindbrain was divided into two horizontal sections which were mounted in paraffin wax. These were then cut into sections 7 to 8 \( \mu \)m thick and stained with hematoxylin and eosin and with a stain combining cresyl violet with Luxol-fast blue. Conventional light microscopy was used to identify irreversible ischemic damage and the results were recorded on diagrams. The procedure was carried out by one of the authors (D.I.G.) who did not know whether the animal was from the control or the lesioned group.

Enzyme Histochemistry

Sections adjacent to those used for autoradiography were stained for maximum activity of the enzyme glycogen phosphorylase, a sensitive marker of ischemic lesions. The histochemical method used was that of Takeuchi and Kuriaki. The preparations were examined to distinguish the pattern of enzyme depletion in order to correlate this with the sites of histologically demonstrated irreversible ischemic damage.

Results

Systemic Parameters

Mean arterial blood pressure remained stable during the 3-hour period of study, and slight elevations observed after lesioning were not statistically significant. Initial mean arterial pressure (\( \pm \) standard error of the mean) was 83 \( \pm \) 5 mm Hg in the control group and 83 \( \pm \) 3 mm Hg in the hemorrhage group. Blood gas analysis was carried out in all animals immediately before injection of blood and at hourly intervals thereafter. All animals in this study had a PaCO\(_2\) between 35 and 40 mm Hg and a PaO\(_2\) greater than 100 mm Hg, and this was maintained throughout the experimental period. The glucose level was measured within 10 minutes of lesioning: the values ranged from 6.6 to 11.0 mM/liter with a mean value of 8.1 mM/liter.

Lesion Topography

Both the animals with a 25-\( \mu \)l injection of blood into the right caudate nucleus and those in the sham-operated control group were studied grossly. The hematomas in the lesioned group had a characteristic pattern: usually the caudate component was small, much of the remainder of the blood having dissected laterally into the white matter of the corpus callosum (Fig. 1). The lesions varied in size and the intracerebral hematomas showed different patterns of surface collections.

Intracranial Pressure Changes

Intracranial pressure rose immediately at the start of induction of the experimental hemorrhage, with a peak occurring within 10 seconds. Following the peak rise there was an immediate decline in ICP but the eventual level remained above that of the control group during the following 3 hours. Cerebral perfusion pressure was...
not substantially reduced during the experimental period (Fig. 2).

**Blood Flow Measurements**

As has been reported previously with this model, at 1 minute after hemorrhage very low levels of CBF (<25 ml/100 gm/min) were identified not only in the area of the blood injection into the caudate nucleus but also remotely in the frontal cortex (Fig. 3a). At 10 minutes, the region with the very low flow had diminished in size. The change was caused by an area of hypoperfusion in the caudate nucleus (Fig. 3b), corresponding with the needle track and resulting hematoma. However, within this area of hypoperfusion, there were few areas with flow of less than 25 ml/100 gm/min, suggesting that flow was returning toward normal (Table 1). At 3 hours, there was minimal evidence of persisting ischemia (Table 1), and a trend toward postischemic hyperemia was evident (Fig. 3c and d). Examples of the autoradiographs are shown in Fig. 4.

**Cerebrovascular Permeability**

All five lesioned animals injected with Evans blue dye demonstrated some extravasation into the caudate nucleus (Fig. 1), in contrast to the five control animals in which Evans blue dye extravasation was limited to the needle track.

Autoradiographic evidence of increased $^{14}$C-AIB crossing the BBB was demonstrated in both the lesioned and the control groups at 25 minutes and 3 hours.
Effects of experimental intracerebral hemorrhage

TABLE 1

**CBF in areas of hypoperfusion in lesioned rats**

<table>
<thead>
<tr>
<th>Brain Region Tested</th>
<th>Time of CBF Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Min</td>
</tr>
<tr>
<td>frontal cortex</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>mid caudate nucleus</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>no. of rats</td>
<td>6</td>
</tr>
</tbody>
</table>

* Cerebral blood flow (CBF) < 25 ml/100 gm/min in the frontal cortex and mid caudate of the lesioned hemisphere. Values are means ± standard error of the means, expressed as a percentage of the total hemisphere. Times indicate interval since placement of the hemorrhage.

There was no significant difference in the intensity of the BBB disturbance between lesioned and sham-operated animals (Table 2 and Fig. 5). On the other hand, the cerebrovascular permeability in lesioned animals at 25 minutes tended to be more extensive than in their respective controls or in the lesioned cases at 1 hour. The product of the number of sections with a focal increase in \( K_i \) and its intensity at the mid caudate nucleus was used to highlight this (Fig. 6). Early lesions were associated with a capillary permeability considerably greater than that in lesions examined at 3 hours.

**Neuropathology**

Irreversible ischemic damage was identified on the side of the injected blood in all lesioned animals. The seven control animals sustained local damage at the site of introduction of the needle but these changes were

---

**Fig. 4.** Carbon-14-labeled iodoantipyrine autoradiographs in the three time groups. **Left Column:** Sections from the lesioned group. The 25-\( \mu l \) lesions are seen as white areas in the right caudate nucleus. At 1 minute, cortical ischemia (white area) is also visible. At 10 minutes, the cortical flow is returning to normal but an area of persisting caudate hypoperfusion is seen in the right caudate nucleus, and cortical flow is no longer at an ischemic level. **Right Column:** Time-matched sham-operated control group. At 1 minute, a minor area of hypoperfusion is seen in the right caudate nucleus at the site of needle insertion. At 10 minutes and 3 hours, the needle track is seen as a faint gray area of hypoperfusion in the mid caudate region.

**Fig. 5.** Carbon-14-labeled aminoisobutyric acid (\(^{14} \text{C}-\text{AIB} \)) autoradiographs. The right caudate nucleus is the lesion site. **Upper Left:** Section obtained 25 minutes after lesioning. There is extensive extravasation of \(^{14} \text{C}-\text{AIB} \) in the right caudate region. **Upper Right:** In this 25-minute control section a small intense lesion is seen in the caudate at the site of needle insertion. **Lower Left:** Section obtained 3 hours after lesioning. The right caudate shows minimal \(^{14} \text{C}-\text{AIB} \) uptake compared to the earlier lesioned brain. **Lower Right:** In this 3-hour control section, there is an area of localized increased \(^{14} \text{C}-\text{AIB} \) uptake in the right caudate nucleus but clearly less than in the control section obtained at 25 minutes.
minimal by comparison to the damage observed in the lesioned animals (Fig. 7).

The distribution of the ischemic damage is shown in Table 3. Structural ischemic cell changes were seen most commonly in the cortex overlying the injection site (present in 55% of the lesioned animals). By contrast, ischemic damage was less common in the caudate nucleus, and none of the lesioned animals had ischemic damage localized only to this region. Each animal with caudate damage also had ischemic changes in the cortex.

**Histochemical Studies**

Sections adjacent to those used for autoradiography were examined histochemically 1, 10, and 180 minutes after the lesions were produced. As early as 1 minute after injection of blood, there was an obvious reduction in phosphorylase activity around the hematomas in all the lesioned animals. The lesion appeared larger at 10 minutes and was unchanged in size at 3 hours. The site of the phosphorylase lesions corresponded with the areas of hypoperfusion identified on the autoradiograms (Fig. 8) but did not correlate with the distribution of the histologically defined ischemic damage (Table 3). On histochemical study, none of the animals demonstrated lesions confined to the cortex: most animals had enzyme disturbance in both the cortex and the caudate nucleus.

**Discussion**

These results display the diversity of the responses to an intracerebral hemorrhage. They indicate that, although the initial vascular effects appear to be of short duration and self-limiting, they result in persistent biochemical and structural changes.

The CBF studies at 1 minute confirmed the pattern of initial ischemia that we have described previously. There was an immediate fall in local CBF in the cortex overlying the hematoma, which was also evident in the ipsilateral frontal cortex. We have previously described the extent of this change which is related in severity to the volume of injected blood, and have demonstrated a less extensive ischemic insult with injection of 25 μl blood than with 50 μl or 100 μl. The reversibility of the CBF and BBB changes in this model may therefore reflect the less severe insult that occurs with a small hemorrhage.

After the initial bleed, there was a gradual return toward normal perfusion; this was observed as early as 10 minutes after hemorrhage and was complete at 3
Effects of experimental intracerebral hemorrhage

hours. Indeed, mild hyperperfusion was seen at 3 hours (Fig. 3c). The distribution curve was shifted to the right of the control graph at 3 hours and also to the right of the other two lesioned-hemisphere curves (Fig. 3d). The duration of the initial ischemia cannot be stated with certainty from the present data, because only three time intervals were studied. It is possible that a focal post-ischemic hyperemia might have occurred. When an ischemic vascular occlusion is opened, hyperemia is often observed during the 1st hour after recirculation.

Increased crossing of the BBB by \(^{14}\)C-AIB was maximum at 25 minutes and showed a tendency to diminish with time. Although this finding was surprising, it seems to be in keeping with a previously reported study of stab injury of the rat cortex, in which the intensity of horseradish peroxidase staining appeared to diminish during the 1st hour after injury.\(^9\) Another explanation might be that the transport mechanisms responsible for the blood-to-brain transfer and trapping of the isotope were impaired due to ischemic damage around the hematoma. However, we did not find evidence of ischemic neuronal damage in this region. Another possibility might be that chemical stimulation by blood or blood products derived from the hematoma led to a transient increase in transfer of isotope to the brain. The removal or inactivation of these blood products might then have led to an apparent reversal of capillary permeability. There is no evidence to support this, although it remains an attractive hypothesis.

Despite the short duration of the changes in blood flow and capillary permeability, structural evidence of ischemic damage was found to be located principally in the cortex overlying the target region. This finding is in accord with the demonstration that 5 minutes of occlusive ischemia can produce histological evidence of ischemic damage to the hippocampus in the gerbil.\(^11\) Although the cortical localization of the present damage could have reflected a more severe or continuing ischemia in the cortex, perhaps due to the vasoconstrictive action of blood, this is unlikely; CBF measurements did not show more prolonged or more severe diminution of flow in this region. It is more likely that the cortex has a lower threshold for ischemic damage than the caudate nucleus and that even the short period of ischemia in our model is enough to cause ischemic damage. Such selective vulnerability has been suggested before in a study of occlusive ischemia in cats.\(^4\)

The principal histochemical change in the caudate nucleus was an area of metabolic disturbance around the hematoma. Unlike the distribution of the ischemic damage, the histochemical lesion was never localized to the cortex alone. This lesion was thus not accompanied by identifiable damage in the caudate, and therefore reflected a more subtle lesion—a biochemical abnormality without structural change.

Both BBB permeability and glycogen phosphorylase lesions have been demonstrated in ischemia in rats due to middle cerebral artery occlusion but not in a hemorrhage model. In occlusive ischemia in gerbils, there was increased phosphorylase activity at a late stage,\(^7\) but we found no evidence of this in our study in rats. This is perhaps because hemorrhage has different effects from occlusive ischemia or, more likely, that the time course of the present study was too short to demonstrate these changes. In a carotid artery ligation model, increased glycogen phosphorylase activity was seen after 6 hours but there was no histological ischemic damage unless an additional hypoxic insult was superimposed.\(^5\) It may be that ischemic damage in the caudate nucleus would have been seen in our model if hypoxia or hypotension had been added.

In these experiments in which a small volume of autologous blood was injected into the caudate nucleus, CBF changes and capillary permeability showed a tendency to resolve with time. Nevertheless, pathological ischemic damage was demonstrated in the cortex. Histological examination revealed that there was a reduction of blood flow in the caudate nucleus, and also disordered metabolic function which did not result in structural damage. This indicates that the potential for recovery in such regions may depend on optimal and lasting restoration of flow with normoxia and normotension. In patients, these added insults may be a key
factor in the development of further brain damage by unfavorably altering the physiological milieu of biochemically deranged areas of the brain.

Acknowledgments

The authors express their gratitude to Anne Semple for typing the paper, and to the Department of Medical Illustration, Southern General Hospital, for their unstinting efforts. Thanks are also due to Mrs. L. Murray for statistical help and advice, and to the technical staff at the Wellcome Surgical Institute.

References


Manuscript received May 29, 1986. This work was supported by a grant from the Medical Research Council of Great Britain.

Address reprint requests to: A. David Mendelow, Ph.D., F.R.C.S., Department of Neurosurgery, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, England.