Experimental Intracerebral Mass: Description of Model, Intracranial Pressure Changes and Neuropathology

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Abstract. In a rodent model designed to replicate the mass effects of spontaneous intracerebral hemorrhage, we have found that there is little change in intracranial pressure (ICP) with microballoons (25 μl and 50 μl in volume) equivalent in size to those lesions which occur with this disorder in man. With larger volumes (100 μl) there is an increase in ICP which is associated with systemic effects on cerebral perfusion pressure (CPP). Neuropathological evidence of ischemic brain damage was found surrounding the mass in all animals, but this was independent of whether the mass was removed or not. These studies suggest that with a mass that corresponds to the size seen most commonly with spontaneous intracerebral hemorrhage in man, focal ischemic brain damage is produced without reduction in global CPP.

Key Words: Cerebellar herniation; Cerebral blood flow; Cerebral hemorrhage; Cerebral ischemia; Cerebral perfusion pressure; Intracranial pressure; Subarachnoid hemorrhage.

INTRODUCTION

Spontaneous intracerebral hemorrhage (SICH) carries a high morbidity and mortality. More than one third of these patients in coma will die (1–3). The prognosis for an intracerebral hematoma resulting from a head injury is also poor. Because of these poor results, much effort has been expended in clarifying the role of surgical and medical therapy for intracerebral hemorrhage. Nevertheless, the most appropriate management remains controversial.

The adverse effects of intracerebral hemorrhage have been attributed to raised intracranial pressure (ICP) (with the resultant decrease in cerebral perfusion pressure), to brain disruption, to disturbances in cerebral microcirculation, to alterations in cerebrospinal fluid (CSF) dynamics and to the effects of the blood cellular elements in the hematoma itself (4, 5).

In previous studies in Glasgow, experimental intracerebral hemorrhage produced a marked reduction in cerebral blood flow (CBF) without a major increase in ICP (2, 6). This reduction in CBF may be due to the physical mass of the hemorrhage or due to biochemical properties in the blood in the hematoma. The purpose of this study was to investigate a mechanical model of an intracerebral lesion in order to clarify the physical, rather than biochemical, factors that might contribute to the pathophysiology of intracerebral hemorrhage.

MATERIALS AND METHODS

General Preparation

Male Sprague-Dawley rats (323–472 g) were used for all studies. All animals had been allowed free access to food and water until the time of the experiment. Anesthesia was induced

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with 5% Halothane in a 70:30% volume mixture of nitrous oxide and oxygen. Once anesthesia had been induced, the animals were then maintained on 0.5–0.75% Halothane throughout the study.

Tracheostomy was performed and the animals were mechanically ventilated using a small animal ventilatory pump. Subsequently, one femoral artery and one femoral vein were cannulated for the continuous measurement of systemic arterial blood pressure, intermittent arterial blood gas determinations and for the administration of fluids. The animal’s temperature was maintained at 37°C by a heating lamp and by measuring the temperature continuously with a rectal probe. Mean arterial blood pressure (MABP) was maintained above 70 mm Hg, PaO₂ above 100 mm Hg and PaCO₂ 35–40 mm Hg. The animals were then placed in a small animal stereotactic frame. A sagittal scalp incision was made and the bregma was exposed. All stereotactic measurements were made from bregma using the Stereotactic Atlas of König and Klippel (7).

### Intracranial Pressure Dynamics

The first series of experiments were designed to discover the changes in ICP and cerebral perfusion pressure in response to intracranial masses of different volumes. Two burr holes were made using a dental drill with continuous saline irrigation to avoid heating. The first cranial opening was over the left hemisphere at a point 1 mm posterior and 1.5 mm lateral to the bregma to allow access to the ventricle for intracranial pressure measurements. The second burr hole was placed over the right hemisphere 1.1 mm anterior and 2.8 mm lateral to the bregma for the introduction of a lesion in the caudate nucleus. The dura mater was then opened at the first burr hole and a fine polythene catheter (1.15 mm external diameter) was placed to a depth of 2.5 mm. The presence of the catheter in the ventricle was confirmed by a brisk increase in ICP in response to abdominal pressure and the presence of cardiopulmonary pulsations on the chart recorder. Once located in the ventricle the catheter was secured in place using dental cement. Arterial blood gas determinations were then repeated and appropriate adjustments made in the inspired tidal volume to ensure that all animals had a PaCO₂ between 35 and 40 mm Hg before the placement of the lesion. Intracranial pressure was measured continuously during the production of the lesion and for the ensuing four hours (h).

### Space-Occupying Lesion

Number 15 microballoons (Ingenor Laboratories, Paris, France) were used to make the lesion. Uninflated they have an external diameter of 0.8 mm and a length of 2 mm. Fully inflated to 100 μl they have an external diameter of 4.5 mm and are 8.5 mm long. Each balloon was mounted on a 25 gauge butterfly® needle (Abbott Ireland Ltd., Sligo, Republic of Ireland) that had been previously filled with Isoopaque Cerebral 280 mg I/ml (Hjegeard and Company, Oslo, Norway). Before mounting the balloon, the sharp end of the needle was removed and the blunt end filed in order to avoid injury to the balloon. The external diameter of the butterfly® needle was 0.5 mm. Each balloon was then expanded and emptied at least three times to ensure its integrity and also to reduce its elasstance so that it would expand smoothly as it was filled. Once this preliminary work was completed, the needle and uninflated balloon were then introduced stereotactically into the right caudate nucleus to a depth of 5.5 mm through the previously placed burr hole. The needle was then sealed in place with dental cement to avoid leakage of CSF or any external herniation of the brain.

After the cement had dried, the balloon was slowly expanded by hand infusion using a Hamilton microsyringe (Hamilton Bonaduz AG, Switzerland) to the desired volume. The expansion was timed to occur over 20–25 seconds (s). There were 17 control (no expansion) animals, 11 with 25 μl injected to inflate the balloon, 10 with 50 μl and 6 with 100 μl. Confirmation that the balloon had expanded was obtained by fluoroscopy (Fig. 1). The balloon remained expanded for four h and ICP and systemic arterial pressures were measured continuously. Arterial blood was sampled hourly to ensure that there were no respiratory abnormalities.
Fig. 1. Lateral skull X-ray of the rat with the intracranial microballoon expanded with 100 μl of radio-opaque material. The extracranial contrast is the dental cement and securing apparatus.

Neuropathology

This series included 15 animals equally distributed between three groups (controls, and two groups with a 50 μl inflation). The first group had the needle and balloon placed in the caudate nucleus for four h but never expanded. The second group had the balloon expanded, again over 20–25 s, to a volume of 50 μl for two minutes (min). During this time fluoroscopic confirmation of the balloon’s expansion was obtained. The Isopaque was then removed but the needle remained in place for the subsequent four h. The third group had the balloon expanded to 50 μl in a similar manner but it remained expanded throughout the four h. The initial studies with a 50 μl mass lesion had shown that a significant compromise of cerebral perfusion pressure did not occur provided that the animal’s blood pressure was maintained. Therefore, only animals with a mean arterial pressure of 80 mm Hg or greater were used in these neuropathological studies. Furthermore an ICP monitor was not inserted, to ensure that the damage observed could be secondary only to the mass lesion.

At the end of four h, the 15 animals were perfusion-fixed with 40% formaldehyde, glacial acetic acid and absolute methanol in a ratio of 1:1:8, vol/vol/vol (FAM) by the method previously described by Brown and Brierley (8). A thoracotomy was performed and a cannula introduced via the left cardiac ventricle into the root of the aorta. The right atrium was incised to allow the fluid that was infused to escape into the chest cavity. At this time, 30 ml of heparinised physiologic saline was infused at the animal’s mean arterial pressure followed by 180 ml of FAM at the same pressure. The animals were decapitated and the head stored in the same fixative overnight. The brain was removed and careful attention paid to any evidence of cerebellar hemisphere herniation below the foramen magnum. The brain was cut into eight slices each 2 mm thick and embedded in paraffin wax. Sections 7–8 μm thick were cut and stained with hematoxylin and eosin (H&E) and with a combination of cresyl violet and Luxol fast blue. Sections were reviewed without knowledge of the animal’s history by one of us.
TABLE 1
Experimental Intracerebral Mass Intracranial Dynamics

<table>
<thead>
<tr>
<th>Physiological variables</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (N = 17)</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>Peak intracranial pressure (mm Hg)</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Intracranial pressure (peak-baseline)</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>Cerebral perfusion pressure</td>
<td>88 ± 3</td>
</tr>
</tbody>
</table>

MABP: mean arterial blood pressure.
* p < 0.001.

(DIG) using conventional light microscopy to identify ischemic brain damage. The areas of ischemic damage were delineated on line drawings of the brain.

Statistics

Comparisons between groups with different volume mass lesions were made for peak intracranial pressure and for the rise in intracranial pressure above baseline. The statistical significance between groups was analysed using an unpaired t-test. A p value of less than 0.02 was used to define significance. Data are presented as mean values ± standard errors of the mean.

RESULTS

Physiological parameters were maintained within normal limits in all studies. The MABP did not differ significantly from the control value in any of the three groups (Table 1).

Intracranial Pressure Study

The intracranial dynamics during the first 30 min are also shown in Table 1. There was not a statistically significant difference between the mean arterial pressures of any group. The only significant difference in peak ICP compared to control was with the 100 µl lesion group (p < 0.001). The control group had a transient elevation of 1 mm Hg over baseline, which lasted less than 15 s, and reflected the initial deformation of the brain immediately before penetration of the needle. There was not a subsequent rise in ICP over the next four h. In the 25 µl and 50 µl volume animals there was also a transient elevation of 3–4 mm Hg respectively in ICP compared to baseline. A representative tracing of the ICP changes with a 50 µl mass is seen in Figure 2. Although these elevations were both significantly different from control (p < 0.001), the extent of the increase did not materially impair CPP. These changes were again transient with the ICP returning gradually toward baseline over four min.

In three animals of the 11 with 25 µl masses and in five of the ten animals with 50 µl masses the ICP had not returned completely to baseline in four min but all were within 3 mm Hg of baseline and continued to decline slowly. As with the control animals, there was no evidence of a late rise in ICP during the four h of recording.
The animals with a 100 \( \mu l \) lesion responded in a more variable manner. The increase in ICP compared to baseline in this group was 16 mm Hg and this was significantly greater than in the 25 \( \mu l \) and 50 \( \mu l \) lesioned animals (p < 0.001). Unlike the animals with smaller volume masses, three of these 100 \( \mu l \) animals had a drop in mean arterial pressure as well as a narrowing of pulse pressure with expansion of the lesion. This resulted in the cerebral perfusion pressure at peak ICP being variable and consequently the CPP did not differ significantly between any of the groups. Also two of these six animals demonstrated a gradual rise in ICP over the last hour of the experiment but this remained below 25 mm Hg.

The comparability of the response in the first three groups in contrast to the 100 \( \mu l \) group can be highlighted by examining the animals with the lowest CPP from each group. In the control group, the lowest CPP was 66 mm Hg. This reflected the animal's MAP of 76 rather than the ICP elevation (peak ICP 10 an increase of 1.5 mm Hg above baseline). The low baseline MABP was also responsible for the low CPP in both the 25 \( \mu l \) and the 50 \( \mu l \) groups of animals. In the 25 \( \mu l \) group the lowest CPP was 71 mm Hg. This animal had a MABP of 82 and peak ICP of 11 mm Hg, which was an increase of only 3.5 mm Hg following expansion of the mass. Likewise in the 50 \( \mu l \) group, the lowest CPP was 62 mm Hg. Again this animal had a low baseline MABP of 70 mm Hg. The increase in ICP was only 4 mm Hg above baseline to a peak of 8 mm Hg. By contrast, the lowest CPP in the 100 \( \mu l \) group was 17 mm Hg. This animal had an increase in ICP of 28 mm Hg to a peak of 38 mm Hg, and the MABP transiently declined to 55 mm Hg from 83 mm Hg. It was the constancy of the response to the 50 \( \mu l \) inflation volume and the absence of any significant decline in CPP that permitted the performance of the neuropathological studies.
without the ICP monitor, provided animals with a MABP of less than 80 mm Hg were excluded.

**Neuropathology**

At the time of the removal of the brain, evidence of raised intracranial pressure as shown by cerebellar herniation was analysed. Neither in the five animals in the control group nor in any of the five animals in which the balloon was only expanded for two min was there evidence of herniation (8). By contrast, three of the five animals in which the balloon was expanded for the entire four h, had evidence of cerebellar herniation. No animal in any group had any evidence of subdural blood secondary to the needle insertion; however, in the two groups in which the balloon had been expanded and then deflated, the cavity created contained a small amount of blood. In none of these cases was there enough hemorrhage for the clot to have acted as a significant mass lesion by itself.

On microscopic examination, the absence of blood in the vessels, the good neuronal morphology, and the absence of cytological artifacts like the "dark cell" change confirmed that perfusion fixation had been adequate (9). In all groups, the areas of ischemic damage were limited to the sensorimotor cortex, through which the needle was inserted, the underlying corpus callosum, the caudate nucleus and the globus pallidus (Fig. 3). In the control group the only histological changes were along the needle tract. By contrast, both the two min expansion and the four h expansion of the mass caused a discrete lesion in the dorsal caudate which extended in some cases into the overlying corpus callosum. The lesions were characterised by pallor of myelin staining and the neuronal changes of the ischemic cell process as described previously in FAM fixed material. There was minimal blood within the remnant of the cavity. The boundary of the lesion was sharply demarcated in the H&E sections from the adjacent normal brain tissue. The amount of structural damage varied slightly from animal to animal, but, as seen in Figure 3, there was not an appreciable difference between the two min and four h lesions. Nonetheless, there was a slight difference in the characteristics of the ischemic area between the two groups. This was seen in three of the five animals with temporary expansion of the 50 μl lesion. In these animals, areas of hemorrhage within the substance of the damaged striatum were consistent with hemorrhagic infarction as seen in an area of reperfusion after local infarction (Fig. 4). The other two animals had appearances that were the same as the animals in which the balloon was expanded for four h.

In none of the animals were there histological abnormalities, either in the contralateral hemisphere or in the hindbrain.

**DISCUSSION**

These studies have shown that balloons of 25 and 50 μl do not produce the major changes in ICP that occur with 100 μl. Ischemic neuronal damage occurs independently of whether the mass is deflated or not. The finding of a relation between the volume of the balloon and an effect on ICP is consistent with the classical understanding of pressure–volume relationships in the central nervous system. Changes in ICP are related to the volume of the mass as well as to the rate of expansion. Weinstein et al (10) suggested that the plasticity of the brain and internal herniation accounted for the variation in ICP. Furthermore, displacement of CSF helped to accommodate an expanding mass. Previously, Langfitt et al (11) showed that the intravascular blood compartment could vary in response to a mass. Small volumes and slow rates of expansion can be accommodated with only minimal changes in
intracranial pressure. Therefore, in experimental studies, the volume of the mass and its rate of expansion are important variables, which can be adjusted to allow the model to replicate the events which occur in man.

The balloon volumes chosen correspond to clinically relevant lesions. The intracranial cavity of the rat is approximately 2 ml as compared to almost 2,000 ml in man. The volumes of balloon we used would represent hematomas of approximately 25 ml, 50 ml and 100 ml in humans. In a series of 132 patients with spontaneous intracranial hemorrhage (SICH), Volpin et al (12) found the average volume to be 53.8 ml. The total flow in one middle cerebral artery (MCA) in man is approximately 150–170 ml/min (13). Consequently, unless all the blood from the MCA went to form the hematoma, the hematoma mass in man could not form more quickly than in the 25 seconds used in this study. As a result, it is not surprising that only a 3–4 mm Hg rise in ICP occurred with the smaller volumes of 25 and 50 μl, respectively. It was only after the brain’s compensatory capacities were overcome with 100 μl that more dramatic changes in intracranial pressure occurred.

Various systemic arterial pressure responses to an intracranial mass have been observed previously. Rosner et al (14) reported a mild decline in blood pressure as

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Fig. 3. Line drawings of the ischemic lesion from each of the animals in the three groups undergoing neuropathologic studies. These are coronal sections at the level of the caudate and are ranked according to size (N = 5 in each group). This represents the level with the largest insult.
Fig. 4. A. 50 µl four h expansion. The border between normal (top) and abnormal brain (bottom) is easily seen as a band of nonhemorrhagic ischemic necrosis at the edge of the lesion. H&E. ×135. B. 50 µl two min expansion followed by four h reperfusion. Again there is a well demarcated border between normal (top right) and abnormal (bottom left). In contrast with A, however, the ischemic lesion is hemorrhagic. H&E. ×135.

the first of three phases in the cardiovascular response to brain injury. They felt the hypotensive response was mediated by the vagal nerves. Similar hypotensive responses have been seen in focal cortical contusion (15) with expanding mass lesions (16) as well as with fluid percussion brain injuries (17). Such vagal responses may have been responsible for the transient decline in systemic arterial pressure in some of our animals with 100 µl masses.
The finding of cerebellar herniation in three of five of the animals in which the 50 \( \mu \)l balloon was expanded for four h despite not having a delayed rise in ICP needs comment. Weinstein et al (10) pointed out that shift with slowly progressive herniation may be one of the compensatory mechanisms for the increased supratentorial volume. In this way ICP could be maintained within normal limits. The phenomenon of brain distortion without elevation in ICP is also seen in man. Because no herniation occurred in the model when the balloon was expanded for only two min, the distortion that we observed is most likely to be the result of secondary mass effects like brain edema. A number of factors may explain the minimal difference between the patterns of structural damage seen after a two min lesion as compared with the four h lesion. One explanation would be that the damage was solely secondary to the destructive shearing effects, which occurred during the brief time the mass was formed. On the other hand, the presence of irreversible ischemic damage indicates that there was a lack of substrate delivery for a more extended time. Patients with intracerebral hemorrhage often have a gradually progressive course (2, 18). Because a two min period of ischemia alone is not enough to result in cell death (19, 20), it is therefore also possible that, although the mass was only expanded for two min, it caused longer lasting effects. These may have included stasis in the vessels surrounding the lesion, although other studies have indicated that this period of stasis would not be long enough to produce this effect (21). A third explanation could be that secondary changes, such as edema precipitated by the expansion of the mass, were responsible for a persisting or ever increasing reduction in cerebral blood flow. If so, then these results mean that these changes occurred independently once the lesion was made, and did not depend on the continuing presence of the mass. This view would lead to the concept that removal of the clot would be useful only in the circumstances that the brain could no longer compensate for the mass effect. That is when ICP begins to rise or CPP begins to fall. The clinical observation that removal of a traumatic hematoma was essential only in patients with elevation in ICP (22) is in accord with this theory. The benefits of operative removal of the clot may therefore lie in effects upon intracranial volume compensation, ICP and CPP rather than on minimizing local brain damage. While there are obvious limitations to the extrapolation of this model to the situation with intracerebral hemorrhage in man, we have controlled the volume and rate of expansion of the mass in these experiments so that the clinical lesion is mimicked as closely as possible.

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