

Intracerebral hemorrhage in the rat: experimental surgical treatments

by

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Intracerebral Hemorrhage in the Rat: Experimental Surgical Treatments

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Mensura Altumbabic

**A Practicum submitted to the Faculty of Graduate Studies of The University
of Manitoba in partial fulfillment of the requirements of the degree**

of

MASTER OF SCIENCE

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ABSTRACT

Intracerebral hemorrhage is associated with considerable mortality and morbidity. The value of surgical therapy is under debate. The purposes of this study were to evaluate, in a rodent model of intracerebral hemorrhage, whether or not aspiration of the blood clot could improve final neurological outcome, and to evaluate whether or not transplantation of fetal forebrain tissue into the hematoma site would improve the final neurological outcome.

Intracerebral hemorrhage was induced in rats by injection of bacterial collagenase into the caudate nucleus. In one group of rats streptokinase was then used to lyse the hematoma 4 hours after hemorrhage induction and the clot was then aspirated. In the transplant study, nine to twelve days after collagenase-induced intracerebral hemorrhage, embryonic day 14 fetal forebrain fragments were transplanted into the hematoma site. Behavioral function was repeatedly evaluated until the rats were sacrificed 7 weeks after collagenase injection in aspiration study, or 10 weeks after grafting. Histology was used to assess overall brain morphology, neuronal loss, astroglial proliferation, and survival of the grafts.

Rats treated with aspirated blood clot performed significantly better than control rats using global motor evaluation tests on Day 1, Day 2 and Day 28. Skilled forelimb testing showed then there was a significant deficit of contralateral forelimb function in both groups, but there was no significant difference between the two groups. Neuronal loss in the penumbra was significantly greater in control rats compared with aspirated rats.

In the transplantation group surviving tissue grafts were located in the residual cavity at the hematoma site. However, comparison of rats with live transplants to control rats with: no transplant, sham transplant, or dead tissue transplant revealed no statistically significant differences in any of the motor behavior tests.

Aspiration of the blood clot after collagenase-induced hematoma improved acute functional outcome and reduced neuronal loss relative to the control group. This is likely due to a reduction in the space occupying effect and possibly improved blood flow in the area surrounding the hematoma. No differences could be detected between the transplantation group and the control.

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LIST OF ABBREVIATIONS

DAB - diaminobenzidine

DMEM - Dulbecco's modified essential medium

GABA - gamma aminobutyric acid

GFAP - glial fibrillary acidic protein

HRP - horseradish peroxidase

ICH - intracerebral hemorrhage

PBS – phosphate buffered saline

MR – magnetic resonance

1.0 REVIEW OF THE LITERATURE

1.1 Introduction

Stroke is defined as a sudden and severe loss of neurological function. Hemorrhagic stroke occurs when a blood vessel or vascular anomaly ruptures, releasing blood into the surrounding brain tissue. Spontaneous intracerebral hemorrhage (ICH) represents one of the most devastating types of stroke ¹, occurring annually in 12-35 person per 100,000 population, and accounting for 9-14% of all strokes ^{2, 3, 4, 5}. More than half of the clinical cases are associated with hypertension in adults aged 55-75 years. The most common sites are the caudate/putamen (striatum), thalamus, cerebellum, and pons ⁶. Other causes of intracerebral hemorrhage include bleeding at the site of ischemic infarct, cerebral vascular malformation, intracerebral aneurysm, cerebral amyloid angiopathy, or trauma.

In general, stroke associated with intracerebral hemorrhage has a worse prognosis than non-hemorrhagic stroke⁷. Mortality following primary intracerebral hemorrhage is high, with the thirty day mortality rate 43-51% ^{4, 5, 8}. Acute neurological deficits are due to direct tissue destruction, the space occupying effect of the hematoma with distortion and ischemic damage in adjacent brain, and diffuse edema in the surrounding tissue ⁹. Recovery following intracerebral hemorrhage is poor, with most surviving patients retaining a considerable functional handicap related to the specific site of hemorrhage ^{4, 5, 8}. Putamen and thalamic hemorrhages are associated with

hemiplegia and hemisensory deficits, while more superficial lobar hemorrhages are more commonly associated with seizures ¹.

1.2 Animal models of hemorrhagic stroke

The development and evaluation of potential therapy for intracerebral hemorrhage relies on use of animal models. Animals models which involve infusion of autologous blood or placement of a small inflatable balloon into the brain have been described ^{10, 11, 12, 13}. These are good models of space-occupying lesions which develop over minutes. Intracerebral hemorrhage can be induced in spontaneously hypertensive rats by ligating the jugular veins and administering tissue plasminogen activator (tPA) and heparin ¹⁴. To achieve better control of the hemorrhage, a rat model has been developed in which intrastriatal injection of bacterial collagenase disrupts the basal lamina of cerebral capillaries and causes bleeding into the brain tissue ^{15, 16}. Evolution of the hemorrhage in this model has been characterized histologically at various times from 10 minutes to 70 days post-injection. Brain edema and behavioral deficits are maximal during the first day following collagenase injection, although locomotor deficits persist for at least 70 days ¹⁷. Del Bigio and coworkers modified this model, infusing a smaller quantity of collagenase together with heparin which enhances the local bleeding. They have found that the hematoma reaches its maximal size approximately 4 hours after injection of collagenase/heparin ¹⁸.

1.3 Pathophysiology of brain damage after intracerebral hemorrhage

In addition to destruction of brain substance by a hemorrhagic event, local cerebral blood flow surrounding a hematoma is compromised ⁹. This is due to decreased autoregulatory mechanisms abilities, vessels spasm, the space occupying effect of the hematoma which distorts adjacent blood vessels, and the diffuse effect of cerebral edema which raises intracerebral pressure and reduces cerebral perfusion pressure. Edema of the surrounding tissue develops due to increasing blood-brain barrier permeability during the first few hours. Increasing intracranial pressure and decreasing cerebral blood flow follow. A rat model of intracerebral space occupying lesion was produced by placing a small (50 μ l) inflatable balloon in the caudate nucleus. Deflation of the balloon after 10 minutes was associated with improved cerebral blood flow and reduced cerebral edema in comparison to animals with permanently inflated balloons ¹⁹.

The penumbra is defined as an area adjacent to the infarct where blood flow and metabolism are compromised. Depending on the circumstances such as blood flow and temperature, the neurons of the penumbra may or may not survive ¹⁸. Medium and large striatal neurons are lost along the edge of the hematoma in non-infarcted tissue (Del Bigio 1996). This effect occurs in the penumbra.

The natural history of an intracerebral hematoma involves degradation of blood and infarcted brain debris. At any brain lesion site, including the site of an intracerebral hematoma, inflammatory cells and proliferating glial cells can liberate a variety of cytokines and growth factors which can be either harmful or

beneficial to injured neurons ²⁰. Del Bigio and coworkers have observed in rat brain that neutrophils begin to infiltrate the surrounding brain at about 12 hours duration and migrate toward the hematoma site. By 48 hours neutrophils infiltrate the hematoma edge and migrate toward the center. This is followed by a wave of monocytes beginning approximately 72 hours after induction of the hemorrhage. Digestion of the hematoma and necrotic brain fragments by monocyte/macrophages occurs during the following days and is largely complete by 2-3 weeks ¹⁸.

1.4 Treatment

1.4.1 Medical

Current management of ICH includes control of systemic hypertension, and treatment or prevention of raised intracranial pressure. Some clinicians have used mannitol and glycerol to reduced intracranial pressure following hemorrhagic stroke ^{21, 22}, but randomized clinical trials have not shown that they improve final neurological outcome ^{23, 24}. Tissue plasminogen activator (tPA), has been shown to be effective for enhancing the clearance of blood from intracerebral hematomas when infused directly into the hematoma ^{25, 26}. Similarly, the proteolytic enzyme streptokinase is of some value for clearing intraventricular blood clots ²⁷.

1.4.2 Surgical

There is much controversy concerning the use of surgery. For removal of the blood clot surgical therapy was first attempted in the early 1900's. Different forms of surgical management, such as open craniotomy, stereotactic injection of thrombolytic agents to facilitate clot lysis and removal, or the endoscopic directed aspiration, have been described as treatment for ICH 28, 29, 30. A meta-analysis of randomized clinical studies in 1997 suggested that immediate surgical removal of hematomas might improve outcome in non-comatose patients less than 60 years of age with a hematoma volume of less than 50 ml. 31, and that problem needs to be studied in a randomized prospective trial.

1.4.3 Experimental transplant of brain tissue for stroke

Fetal brain tissue transplantation is a method under investigation for promoting brain recovery following experimental ischemic stroke 32. There are also data suggesting that purified immature astrocytes can encourage regrowth of injured axons when grafted into a brain lesion 32, 33, 34. The mechanism by which regeneration is enhanced is not entirely understood, but immature astroglial cells are believed to produce trophic factors which promote neuronal survival and regeneration after brain injury 35. Transplantation approaches have not been studied in models of hemorrhagic stroke involving the basal ganglia.

In two experiments, striatal ischemia induced in rats by occlusion of the middle cerebral artery was followed 2 or 8 weeks later by transplantation of fetal

forebrain cells. Behavioral improvement and increased production of GABA was observed as early as 4 weeks later and persisted for up to 1 year^{36, 37}.

1.5 The purpose of this study was to test the following hypotheses:

1. Aspiration of collagenase-induced hematoma from rat brain is associated with improved neurological outcome.

2. Transplantation of fetal rat forebrain tissue into the hematoma site is associated with improved neurological outcome in rats with collagenase-induced striatal hematoma.

2.0 MATERIALS AND METHODS

2.1 Experimental ICH in rat

All experimental procedures were done in accordance with guidelines of the Canadian Council on Animal Care. Young adult male Sprague-Dawley rats weighing 175-250 g were used. Each rat was anesthetized with pentobarbital (50 mg/kg ip) and placed in a stereotactic frame (David Kopf Instruments). Through a hole drilled in the skull, a 30-gauge needle was introduced into the caudate nucleus (3 mm lateral to midline, 0.02 mm anterior to coronal suture, depth 6 mm below the surface of the skull), and 1.4 μ l of saline containing 0.3 U collagenase (Type IV, Sigma Chemical Co., St. Louis) was infused over 7 minutes. After infusion the needle was left in place for 3 minutes, then removed. The bone hole was sealed with bone wax, the scalp wound sutured, and the animal placed in a warm box with free access to food and water.

Bacterial collagenase was injected into the right or left striatum based on rat's preference to grasp with left or right forelimb in staircase apparatus during pre-training period.

2.2 Aspiration study

Forty-four behavioral trained and tested rats underwent histopathological examination seven weeks after hemorrhage. Fourteen other rats were killed 24 hours after ICH for brain water determinations. After ICH every second rat was selected for aspiration of the blood clot. Four hours after collagenase injection the rat was reanesthetized with pentobarbital (50 mg/kg ip) and again placed in the

stereotactic frame. Using the same stereotactic coordinates, streptokinase (3 μ l; 1000 U/ μ l, Sigma) was injected by 27-gauge needle into the hematoma center. One hour later, aspiration was accomplished by application of gentle suction with a syringe attached to a 25 gauge needle placed at the same stereotactic coordinates. The volume of aspirated blood was measured.

2.3 Transplant study

Thirty-one behavioral trained and tested adult male rats, housed individually in reversed day/night lighting conditions, were used in the transplantation study. Under pentobarbital anesthesia intrastriatal hematoma was produced as described above. Nine to twelve days after hematoma induction the rats were randomly assigned to one of four groups: untreated hematoma (n=5), hematoma followed by transplantation of live tissue (n=16), hematoma followed by transplantation of microwave-killed tissue (n=5), or hematoma followed by sham transplantation (needle insertion only for 30 seconds) (n=5). Four locally mated female rats, three 14 days postcoital and one 15 days postcoital, were used as donors. They were anaesthetized with pentobarbital, the uterus was removed, and embryos were isolated under aseptic conditions. Cranial membranes were stripped and the telencephalic vesicles were pinched off and placed in DMEM culture medium (Gibco BRL). Forebrain tissue fragments were incubated with 18 μ M Hoechst 33342 (10 μ g/ml bisbenzimidazole trihydrochloride; Sigma) for 1 hour at 37°C in the dark to allow identification of transplanted cells in the host brain³⁴. Tissue was washed with 0.9% sterile saline and coated with sterile charcoal as a site marker. Two pieces of embryonal

forebrain tissue (approximately 0.2 mm diameter) were injected into hematoma site through a glass cannula using the same stereotactic coordinates as described above.

2.4 Behavioral Testing

In the aspiration and transplant study, several tests were used to assess general motor behavior (see below) and skilled forelimb function³⁸. This testing required training prior to induction of ICH. Rats had free access to food and water during first two days after arrival from the supplier. The rats were housed in pairs in standard plastic boxes with a 12 h day/night cycle. During the following seven days rats were fed 8-15 g of standard laboratory chow to reduce body weight to 85-90% of the initial weight. Staircase pre-testing was performed twice a day with a time interval of at least four hours. The rat was placed in a clear plastic box with a food-baited staircase on either side. On each staircase were seven steps, each with a well containing three 45 mg pellets (P.J. Noyes Co. Inc., Lancaster NH). The number of food pellets reached and eaten in 20 minutes was counted. When a plateau in the number of pellets eaten in 20 minutes was reached, the top well was no longer baited with pellets, because the top well could be reached with the tongue. An additional 4 to 6 trials were used to calculate mean pre-training value for each side. If side-to-side difference was > 4 on all the final trials, the side on which rat collected more pellets was designated its "preferred" side. Animals were then allowed free access to food for two days prior to surgery. The hematoma was induced in the dominant brain hemisphere in rats with a preferred side.

Global behavior was evaluated in each rat 1, 3, 5, 7, 11, 14, 17, 21 and 28 days after collagenase injection. The tests included (i) spontaneous ipsilateral circling, graded from 0 (no circling) to 3 (continuous circling); (ii) contralateral hindlimb retraction, measuring the ability of the animal to replace the hindlimb after it was displaced laterally by 2 to 3 cm, graded from 0 (immediate replacement) to 3 (no replacement); (iii) beam walking ability, graded 0 for a rat which readily traverses a 2.4 cm wide 80 cm long beam, to 3 for a rat unable to stay on the beam for 10 seconds; and (iv) bilateral forepaw grasp, which measures the ability to hold onto a 2 mm diameter steel rail, graded 0 for a rat with normal forepaw grasping behavior, to 3 for a rat unable to grasp with the forepaws. The neurological deficit score was taken as the sum of scores from all four tests (maximum possible deficit score 12).

The skilled forelimb test was used to detect more subtle deficits at later time points. Beginning 28 days after ICH the rats were fed 10-20 g standard laboratory chow to decrease the body weight to 90% of the free feeding level. They were then evaluated once daily in the staircase apparatus for three weeks. The top well of the staircase apparatus was not baited. The number of food pellets eaten in 20 minutes on each side was counted (maximum possible 18).

In the transplant study, prior to ICH the rats underwent a 16 trial training session in the use of a staircase feeding apparatus. Global behavior was evaluated using three tests in each rat 1, 3, 5, 7, 14, and 21 days after induction of ICH. The tests included observation of spontaneous circling toward the side of the lesion, contralateral forelimb reaching, and beam walking ability as previously described. A deficit score (maximum 9) was assigned based on these tests. In the 4th week after transplantation, rats were placed on a 6 cm diameter cylinder

rotating at 20-25 rpm. Time spent on the cylinder was measured (maximum 3 min). The same day, square pieces of tape (6 x 6 mm) were simultaneously placed on the lateral aspect of both forelimbs and the time necessary to remove the pieces of the tape by mouth was measured ³⁹. Rats were evaluated daily in the staircase feeding apparatus for 4 weeks beginning the 3rd week after transplantation. The mean number of food pellets eaten in 20 minutes on each side was counted (maximum possible 21 per side) during the 7th week after transplantation. Spontaneous activity was measured twice in the 8th week after transplantation using an infrared beam activity monitor (65 cm diameter opaque drum; Lehigh Valley). Rats were placed in the apparatus for 10 min and number of beam interruptions was registered ⁴⁰. To assess lateralizing tendency, rats were suspended by the tail 10 cm above a surface for 30 seconds. The number of swinging episodes to each side was counted; rotational swinging in one direction greater than 70% of the time is indicative of unilateral neurological deficit ⁴¹. Ten weeks after transplant, apomorphine-induced (0.1 µg/kg s.c.) circling was assessed with video recording for 15 minutes. The preference (if any) was expressed as the percent rotations toward the lesion side.

2.5 Brain water content analysis

Fourteen rats were used for this experiment. Six rats had collagenase - induced hemorrhage, and eight rats had collagenase - induced hemorrhage followed by aspiration as described above. Twenty-four hours after ICH motor behavior tests were done, then each rat was killed by pentobarbital overdose. The

brain was quickly removed and placed on a cooled surface, then the cerebellum and brain stem were removed. The cerebrum was divided into hemispheres, and each hemisphere was coronally cut into three parts; the first cut was through the needle entry site and the second through the midpoint of the posterior remnant. Each section was weighted, wrapped in pre-weighed aluminum foil, dried for three days in an oven at 110°C, then weighed again. Water content was calculated as the percentage change between wet weight and dry weight.

2.6 Histological Examination

In the aspiration study, seven weeks after collagenase injection each rat was reanesthetized and perfused transcardially with 300 ml of cold 4% paraformaldehyde in 0.1 mole/L phosphate buffered saline (PBS). The brain was removed and stored in the same fixative. Fixed brains were cut coronally through the needle entry site (identifiable on the brain surface), as well as 2 mm anterior and 2 mm posterior to that plane. Brain slices were dehydrated and embedded in paraffin. Sections (5µm) were cut and each tenth section from the rostral to the caudal portion of the residual hematoma cavity was stained with hematoxylin and eosin.

All sections were inspected macro- and microscopically to determine anatomical structures involved in the hematoma site. According to the part of striatum involved, hematomas were divided in medial, lateral, or whole striatum location. Extension of hematoma into the internal capsule was qualitatively graded: 0 - no extension; 1 - extension in anterior limb; 2 - extension into posterior

limb. Extension of hematoma into the thalamus was similarly graded: 0 - no extension; 1 - focal calcium deposition and cell loss; 2 - residual cavity.

A "camera lucida" drawing was used to quantify brain damage on the coronal slice with maximum hematoma diameter. Onto a sheet of paper, the ipsilateral cortical injection site, hematoma cavity, residual striatum, and ventricle were traced, as were the contralateral striatum and ventricle. Computerized planimetry (MICRO-PLAN II Laboratory Computer System, INC. Cambridge, MA) was used to measure the traced areas. Side-to-side differences were compared. Striatal area loss was calculated as the percent difference in area between contralateral and ipsilateral striatum.

Medium-sized striatal neurons, which could be identified on the basis of nuclear morphology (round nucleus diameter 15-25 μm with prominent nucleolus), were quantified at the coronal level of the maximum hematoma diameter as previously described^{18, 42}. With a square ocular graticule and x250 ocular magnification (objective magnification x20), neurons were counted in three fields (each area 400x400 μm) immediately adjacent to the hematoma site, avoiding areas with large blood vessels. Three anatomically comparable fields in the contralateral caudate nucleus were assessed in the same manner. The difference between the sums from each side was used as an index of relative neuronal depletion in striatal tissue adjacent to the hematoma.

GFAP immunohistochemical labeling was performed on sections at the coronal level of the maximum hematoma diameter. Sections were quenched with 3% hydrogen peroxide in methanol for 10 minutes, incubated with 20% goat serum for 30 minutes at room temperature, then the primary polyclonal GFAP antibody (Dako, dilution 1/400) was applied overnight at 4° C. Secondary

biotinylated goat anti-rabbit (1/300) was applied for 1 hour at room temperature, followed by streptavidin HRP and then DAB. Reactive astrocytes densities were compared between the ipsilateral and contralateral cortex and internal capsule and assigned a grade of 0 if the same, 1 if slightly greater, and 2 if much greater on the side of the hematoma. Reactive gliosis extension adjacent to the residual cavity was measured with a calibrated ocular graticule.

Rats brain were prepared for histological study 10 weeks after transplantation, by an identical technique to that used for the aspiration study. Brains were embedded in paraffin for histological analysis. Paraffin sections were stained with hematoxylin and eosin or coverslipped unstained for viewing by epifluorescence microscopy using ultraviolet illumination.

2.7 Data analysis

Data were analyzed using StatView Version 4.1 (Abacus Concepts, Inc.; Berkeley, CA). Z-score histograms were used to determine if the data were distributed normally. For the skilled forelimb test the mean post-hematoma score was calculated for each week (5 trials) for each side separately. The mean pre-hematoma and post-hematoma values were compared. Normally distributed data (behavior, areas of residual cavity, ventricle, striatum, cortical damage, neuronal count, water content) were analyzed by Student's t-test, to compare the aspiration group and control. Correlation coefficient or regression analysis were used to assess the relationship between morphologic features and behavioral outcomes. The Kruskal-Wallis non parametric test was used to assess the relationships between hematoma location or extension into the internal capsule

and the functional deficits. All behavioral data were quantified and analyzed by ANOVA with post-hoc Bonferroni-Dunn intergroup comparisons or regression analysis as appropriate. Differences were considered significant at $p < 0.05$.

3.0 RESULTS

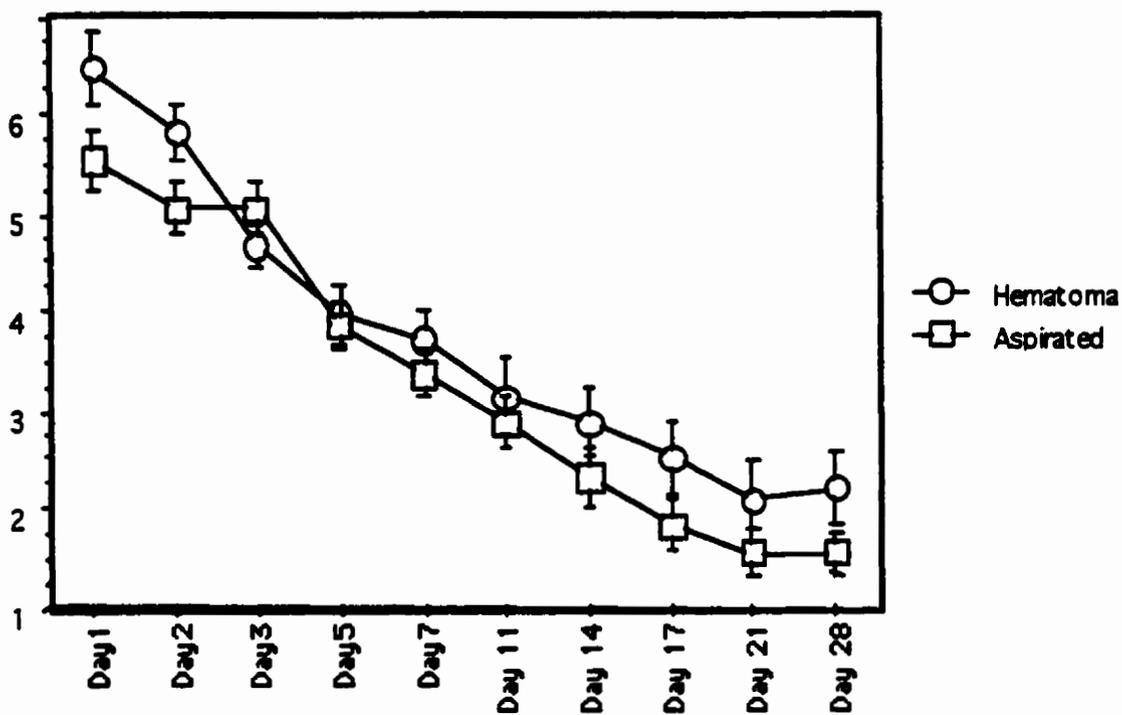
3.1 Aspiration study

The data from this experiment have already been published in a peer-reviewed journal ⁴².

Six rats were excluded prior to surgery because they refused to eat during staircase training. No rats died immediately following surgery. Two rats were euthanized 4-5 weeks after ICH following unexpected weight loss. Histological analysis showed a large abscess in the cortex and striatal region of each rat. One rat was excluded because the bacterial collagenase had been injected into the septal region. For analysis, there were 18 rats with naturally evolving hematomas and 17 rats with aspirated hematomas. The volume of aspirated blood ranged from 20 to 100 μ l.

Motor deficit scores during the first four weeks after ICH are shown in **Figure 1**.

Figure 1. Line graph showing motor deficit scores (mean \pm SEM) in rats with naturally evolving hematoma (circle) and rats whose hematoma had been aspirated (squares). The scores are significantly better for the treatment group on Day 1, Day 2 and Day 28 ($P < 0.03$) (one-tailed Student's *t* test)



The number of food pellets eaten reached a plateau after 8 - 10 trials in the training period. Four rats had constant preference on the right side, five rats had preference for the left side. The plateau was reached in 5 trials during post ICH testing. There were no differences in performance between the groups prior to ICH. The limb ipsilateral to the hematoma exhibited no loss in performance following ICH. There was a significant decline in function of the forelimb contralateral to the hematoma, but there was no difference between treated and untreated rats. Results of the skilled forelimb testing are shown in **Table 1**.

The cortical lesion was larger and the ipsilateral ventricle was less dilated in the treated group. The anterior limb of the internal capsule and portions of the ventroposterior and ventrolateral thalamic nuclei sustained some damage in most rats. Localization of hematomas, anatomical structures damaged by ICH, and relative sizes of the residual lesions are shown in **Tables 2 and 3**.

Table 1. Skilled forelimb testing in rats with intracerebral hematoma.

| | Contralateral to hematoma | | Ipsilateral to hematoma | |
|-------------------|---------------------------|----------------|-------------------------|----------------|
| | Pre - ICH (a) | Post - ICH (b) | Pre - ICH (a) | Post - ICH (b) |
| Untreated control | 11.1 ± 0.6 | 6.0 ± 1.0 * | 10.7 ± 0.6 | 10.0 ± 1.0 |
| Treated | 12.1 ± 0.4 | 6.0 ± 0.9 * | 10.6 ± 0.6 | 11.2 ± 0.7 |

a) number of pellets eaten (daily mean ± SEM) during pre-training prior to ICH;

b) number of pellets ate (daily mean ± SEM) during last 5 days of testing.

* p<0.05 vs. pre - ICH

Table 2. Brain structures damaged by intracerebral hematoma and relationship to skilled forelimb performance.

| Structure damaged (a) | Untreated control (n=18) | Treated (n=17) (b) | Number of pellets eaten in relation to contralateral brain damage (c) |
|---------------------------------|---------------------------------|---------------------------|--|
| Medial striatum | 11 | 10 | 7.2 ± 0.8 |
| Lateral striatum | 3 | 3 | 4.7 ± 1.5 |
| Whole striatum | 4 | 4 | 2.9 ± 1.1 * |
| Internal capsule damage grade 0 | 1 | 1 | 8.2 ± 3.8 |
| Internal capsule damage grade 1 | 13 | 12 | 6.1 ± 0.8 |
| Internal capsule damage grade 2 | 4 | 4 | 4.3 ± 1.3 |
| Thalamus damage grade 0 | 2 | 1 | 5.6 ± 2.1 |
| Thalamus damage grade 1 | 13 | 12 | 6.7 ± 0.7 |
| Thalamus damage grade 2 | 3 | 4 | 1.6 ± 0.6 ** |

a) Damage grades are explained in Methods.

b) The pattern of brain damage was not different between the treated and untreated groups.

c) Results from skilled forelimb testing in staircase apparatus for combined control and treatment groups. The number of pellets (mean ± SEM) retrieved by the forelimb contralateral to the ICH was subdivided according to the damage location or grade specified. Within the subheadings of striatum, internal capsule, or thalamus the three levels of damage were compared. * $p < 0.025$ whole striatum injury vs. medial striatum only injury; ** $p < 0.02$ thalamus grade 2 damage vs. thalamus grade 0 damage, (Kruskal-Wallis test).

Table 3. Relative size of brain structures and damage areas.

| Site (a) | Untreated control | Treated |
|---------------------------|-------------------|-------------|
| Hematoma Cavity | 1.8 ± 0.3 | 1.7 ± 0.3 |
| Cortical Damage | 0.9 ± 0.1 | 1.5 ± 0.2 * |
| Striatum - Ipsilateral | 4.4 ± 0.5 | 4.0 ± 0.5 |
| Ventricle - Ipsilateral | 4.6 ± 0.6 | 3.1 ± 0.4 * |
| Striatum - Contralateral | 9.7 ± 0.7 | 9.8 ± 0.5 |
| Ventricle - Contralateral | 1.9 ± 0.2 | 1.3 ± 0.2 |

a. All sizes are reported as mm² (mean ± SEM), as measured by planimetry on a single coronal histological section at the level of the maximal hematoma diameter.

*p<0.05 vs. untreated group (two tailed t test)

There were no correlations between the functional performance on day 1 and hematoma location in the striatum, hematoma extension into thalamus or internal capsule, or the volume of blood aspirated in treated rats. However, the final skilled forelimb performance depended on the hematoma location within striatum and extension of injury into the thalamus (Table 2). Rats with medially placed hematomas had the least deficit. By regression analysis, there were no significant relationships between the final skilled forelimb performance and area of cortical damage, size of residual striatal cavity, or volume of blood aspirated in treated rats.

The mean neuronal count in contralateral sides in untreated rats was 154.4 ± 9.4 and in treated rats was 153.5 ± 9.0 , while the mean of the neuronal count in the ipsilateral side was 99.6 ± 9.3 in untreated and 145.0 ± 11.0 in treated rats. Striatal tissue surrounding the hematoma in untreated rats exhibited significantly greater neuronal loss than in treated rats ($p < 0.0014$). There was no difference in the absolute neuronal count in the contralateral striatum between the two groups. Reactive gliosis extended on average $416 \pm 56 \mu\text{m}$ from the residual cavity in untreated rats, and $296 \pm 40 \mu\text{m}$ in treated rats ($p < 0.04$ one tailed Student's t-test). There was no significant difference in cortical or external capsule gliosis between two groups.

Water content, 24 hours after collagenase injection (shown in Table 4) was increased in the cerebrum ipsilateral to the hematoma compared to the contralateral side in both groups. However, there was no difference in water content between treated and untreated groups.

Table 4. Water content in brain slices 24 hours following intracerebral hemorrhage ^(a)

| Location | Untreated control | Treated |
|-------------------------|--------------------------|----------------|
| contralateral anterior | 80.6 ± 0.3 | 80.9 ± 0.5 |
| contralateral middle | 79.8 ± 0.3 | 79.5 ± 0.7 |
| contralateral posterior | 79.8 ± 0.4 | 79.7 ± 0.3 |
| ipsilateral anterior | 81.2 ± 0.3 * | 81.1 ± 0.4 * |
| ipsilateral middle | 80.7 ± 0.3 * | 80.9 ± 0.4 * |
| ipsilateral posterior | 80.0 ± 0.4 | 79.9 ± 0.3 |

a. Percent water content calculated by wet weight - dry weight differences, mean ± SEM.

* The ipsilateral anterior and middle sections include the hematoma site and had significantly greater water content ($p < 0.05$) than the corresponding contralateral sections.

3.2 Transplant study

The data from this experiment have already been published in a peer-reviewed journal ⁴³.

Three rats were excluded prior to surgery because they refused to eat in the staircase apparatus. Five rats with live transplants were sacrificed or died prior to completion of behavioral testing.

Histological analysis of the surviving rats showed that the hematoma in untreated and sham transplant rats was totally resorbed. Only a collapsed residual cavity with hemosiderin-containing macrophages and reactive astrocytes remained, similar to that previously described ¹⁸. The ipsilateral striatum was atrophic and the ipsilateral ventricle was enlarged. In rats with microwave-killed transplants, particles of charcoal were seen in or near the residual cavity but the pathological changes did not otherwise differ.

Histologically viable donor tissue consisting of matured glial tissue and large neuron cells were seen in 8 of 16 rats that had received live grafts. The host-transplant margins were usually apparent because of differences in cell and fiber orientation, however, there were no specific anatomical barriers between the two. Donor cell nuclei exhibited blue fluorescence under ultraviolet excitation. Five transplants were large, from 4 to 8 mm diameter. In seven of eight rats, the transplant extended from the residual hematoma site into the lateral ventricle. Growth of transplant tissue into the intraventricular foramen was associated with contralateral ventricle enlargement in 4/8 rats. The largest transplants compressed the surrounding brain tissue. There was minimal glial reaction at the transplant-host interface.

Comparison of rats with successful live transplants to control rats in other groups, revealed no significant differences in any of the behavioral scores, assessed before or after transplantation at any time point (Table 5). There was no relationship between transplant size and motor performance.

Table 5. Motor behavior testing in rats with intracerebral hemorrhage and brain fragment transplants (a).

| | Untreated control (n=5) | Transplant sham (n=5) | Transplant dead (n=5) | Transplant live (n=8) |
|---|----------------------------|--------------------------|--------------------------|--------------------------|
| Deficit score Day 1 post- ICH (pre-transplant) | 5.0 ± 2.1 | 4.7 ± 2.1 | 5.4 ± 1.0 | 3.4 ± 2.1 |
| Deficit score day 21 post-ICH (b) | 1.7 ± 1.3 | 2.1 ± 1.5 | 2.1 ± 1.2 | 1.6 ± 1.5 |
| Contralateral feeding pre-ICH (c) | 11.6 ± 1.9 | 10.8 ± 1.9 | 12.9 ± 1.0 | 10.6 ± 2.2 |
| Contralateral feeding post-ICH (d) | 10.8 ± 1.3 | 6.9 ± 6.9 | 8.1 ± 3.7 | 10.3 ± 3.6 |
| Rotating beam agility (e) | 13.8 ± 11.4 | 5.3 ± 2.4 | 36.5 ± 43.0 | 13.0 ± 8.4 |
| Tape test (f) | -28.2 ± 69.6 | -21.1 ± 86.8 | 38.1 ± 86.2 | 8.3 ± 6.9 |
| Spontaneous activity (g) | 628 ± 170 | 663 ± 196 | 682 ± 190 | 616 ± 165 |
| Apomorphine-induced rotation (h) | 50.6 ± 35.0 | 63.8 ± 25.0 | 63.4 ± 37.6 | 58.8 ± 5.1 |

a. All data are expressed as mean ± SDM. There were no statistically significant differences between any of the groups (ANOVA). Details of behavioral tests appear in the methods.

b. Deficit score week 2 post-transplant

c. Number of pellets eaten in staircase apparatus during pretraining period prior to hemorrhage.

d. Number of pellets eaten in staircase apparatus; week 7 post-transplant.

Table 5 legend (continued)

- e. Time (seconds) on rotating beam until falling; week 4 post-transplant.**
- f. Time (seconds) delay removal of tape from forelimb ipsilateral to ICH until contralateral tape removed; week 4 post-transplant. A negative value indicates that tape from the affected forelimb was removed first.**
- g. Number of infrared beam interruptions in 15 minutes; week 8 post-transplant.**
- h. Percent of rotations toward side contralateral to ICH; week 10 post-transplant.**

4.0 DISCUSSION

4.1 Benefit of hematoma removal post-ICH

There is considerable controversy regarding the value of surgical removal of the blood clot over conservative therapy for spontaneous intracerebral hemorrhage. Although many studies have been reported, most are considered inadequate to reliably quantify the risk and benefit of surgical treatment. A prospective trial comparing open craniotomy within 12 hours of onset of ICH symptoms to best medical therapy showed an "early mortality" benefit from surgery with a mortality rates of 6% in the surgical group, and 24% in the non-surgical group within the first month. There were no differences in long-term benefit in this investigation at six months (Morgenstern 1998). Two recent independent reviews of the literature with meta-analysis assessment 31, 44 determined that there are only four randomized trials of surgical treatment worth considering 28, 45, 46, 47. Both of these groups of authors concluded that there was insufficient information concerning the safety and efficacy of surgery, and that more information was needed from a multi-center randomized trial to determine whether indeed some patients with ICH may benefit from surgical therapy.

We wished to determine whether or not the aspiration of a collagenase-induced hematoma could improve the final outcome in rats and, if so, by what mechanism. Pilot experiments using magnetic resonance (MR) imaging before and after hematoma aspiration showed that only a portion of the center of the hematoma could be removed (unpublished data; NOTE: MR was not used for the

experiment because the MR spectrometer was not functional during the period of study).

Intracerebral hemorrhage causes brain damage by multiple mechanisms. Direct tissue destruction by the hemorrhagic event and dissection of blood along tissue planes occurs immediately. Damaged cells and axons in the path are unlikely to be saved by any intervention. As a result of the space-occupying effect of the hematoma, there is compromise to local blood flow in surrounding tissue. This has been shown by a variety of experimental methods following inflation of balloons or injection of autologous blood into brain ^{9, 19, 48, 49, 50, 51}. Our preliminary experiments using MR blood perfusion imaging also indicate that blood flow is reduced in an area much larger than the hematoma itself (unpublished data 1997). These observations suggest that ICH has a penumbral region, similar to that adjacent to ischemic brain damage in which blood flow is reduced and neuronal function and survival is compromised ⁵². However, Qureshi and coworkers have indicated that there is no evidence to support the existence of an ischemic penumbra in the first 5 hours after autologous blood injection ICH in dogs⁵³. It is important to note the finding that a 50 μ l balloon implanted into rat caudate nucleus was associated with recovery of cerebral blood flow ⁴⁹. This might explain why we observed improved neuron survival and reduced reactive gliosis in the striatum adjacent to hematomas that had been treated by aspiration.

Acute behavioral improvement in the aspiration experiment did not appear to be a result of the reduction of brain edema which develops in the first few hours and peaks at 24 to 48 hours ¹⁸. The edema associated with autologous

blood injection into the brains of rats and pigs has a similar time course development^{51, 54}. Rosenberg and coworkers have successfully reduced brain water in rats with collagenase-induced ICH using a variety of drugs, but outcomes were measured only at 24 hours and behavior was not assessed in detail ^{55, 56, 57, 58}. In contrast, Wagner and coworkers documented reduced peri-hematoma edema in the pig ICH model following tissue plasminogen activator-assisted evacuation ⁵⁹.

Despite our documentation of acute behavioral improvements and reduced neuronal death in the striatum, surgically treated rats did not exhibit any late benefit with regard to skilled forelimb performance. This is likely a function of the brain structures that were damaged by the hematoma. Most of the hematomas extended into the internal capsule and thalamus. For successful reaching and grasping in the staircase test, the corticospinal tract, basal ganglia, and ascending sensory pathways need to be intact ^{60, 61, 62}. Axonal damage in the internal capsule and thalamus would be expected to affect these pathways and it is very unlikely that axonal damage would be reduced by aspiration of blood from the striatum. It is also worth noting that hematomas involving either the lateral or entire striatum were associated with greater disability than those with only medial damage. This has been shown directly in other experiments where lesions of the lateral striatum produced severe and chronic impairment of movement initiation, forelimb reaching amplitude, and postural synergism ⁶³. Damage to the medial striatum produced mild or no impairment of forelimb reaching. In rat, the lateral striatum is more directly involved with motor function.

4.2 Lack of benefit of fetal transplant post-ICH

Fetal striatal neurons have been shown to survive and integrate when grafted into the striatum, in some cases establishing functional connections with host neurons ³³. Early improvement following transplantation of fetal neurons has been attributed to production of trophic substances and/or neurotransmitters ³². The fact that we did not see any functional improvement associated with transplantation could have several explanations. It is possible that the behavioral tests used in this study were not able to detect minor improvements. However, with the range of tests performed one would expect to have seen improvement in at least one parameter. Our animals were tested only up to 10 weeks after grafting and we cannot exclude the possibility that a longer survival period might have been associated with improved functional outcome when true neuronal connections became established. However, improvements following ischemic striatal injury have been observed 4 weeks after transplantation ³⁷, therefore we felt justified in discontinuing this experiment at 10 weeks. The donor cells may not have produced any usable neurotransmitters or trophic agents capable of supporting the host neurons. This is perhaps not surprising when one considers that ICH is associated with considerable mechanical disruption of tissue which, in contrast to selective neuronal death, would not be compensated for by a transplant.

One problem with this experiment was the uncontrolled growth of fetal brain tissue. Large grafts behaved as tumors, compressing surrounding brain

tissue and causing hydrocephalus. Similar complications have been reported in a patient who received a fetal mesencephalon transplant for treatment of Parkinson's disease ⁶⁴. Although the final graft size did not have a linear influence on outcome within the transplant group, we cannot exclude it as an explanation for the lack of benefit.

Borlongan reviewed the literature concerning transplantation of fetal tissue as a method for improving brain recovery in animal models of stroke. At present the results are far from conclusive ³². Some investigators suggest that transplants are only beneficial in animals cared for in an enriched environment ^{65, 66} which can stimulate brain plasticity and is associated with increases in cortical thickness, protein content, and dendritic branching ⁶⁷. Standard laboratory housing conditions provide limited sensorimotor stimulation and opportunity for locomotor activity. However, the frequent testing of our rats is, in itself, a form of environmental enrichment.

5.0 CONCLUSIONS

We conclude that surgical aspiration of collagenase-induced intracerebral hematomas from rat brains improves acute functional deficit through reduction of the space occupying effect of the hematoma and consequent reduction of intracerebral pressure. Acutely damaged axons probably do not benefit by surgical treatment. Late neuronal survival in the striatum surrounding the hematoma was also improved, possibly due to improved local cerebral blood flow. Thus it appears that intracerebral hematomas may be associated with a penumbra similar to that associated with ischemic tissue. Whether this aspect of local tissue benefit is clinically relevant is not known, but it does suggest that drugs to treat cerebral edema and neuronal ischemia combined with surgical treatment in select patients might be beneficial.

We conclude that fetal brain transplantation into the site of collagenase-induced striatal hemorrhage in rats does not appear to be beneficial. This does not exclude the possibility that transplantation of purified neural stem cells or that if more controlled growth of the fetal brain tissue were achieved, the transplantation might be helpful in recovery after intracerebral hemorrhage.

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7.0 APPENDICES

Intracerebral Hemorrhage in the Rat

Effects of Hematoma Aspiration

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Background and Purpose—Deep intracerebral hemorrhage is associated with considerable mortality and morbidity, but the value of surgical therapy is debatable. The purpose of this study was to evaluate whether aspiration of the hematoma in a rodent model of intracerebral hemorrhage could improve final neurological outcome.

Methods—Intracerebral hemorrhage was induced in 2 groups of rats by injection of bacterial collagenase into the caudate nucleus. In 1 group of rats, streptokinase was used to lyse the hematoma 4 hours after hemorrhage induction, and the clot was then aspirated. Behavioral function was evaluated repeatedly until the rats were killed 7 weeks after collagenase injection. Histology was used to assess neuronal loss, astroglial proliferation, and overall brain morphology. In a second experiment, brain water was measured at 24 hours.

Results—The treated rats performed significantly better than controls on a motor-behavior evaluation on days 1, 2, and 28 after aspiration. Skilled forelimb testing performed for 3 weeks after the global behavior evaluations showed a significant deficit of contralateral forelimb function in both groups, but there was no significant difference between the 2 groups. Neuronal loss in the perihematoma striatum was significantly greater in untreated compared with treated rats. In most rats, structural damage extended into the internal capsule and thalamus.

Conclusions—Aspiration of the hematoma after collagenase-induced hemorrhage slightly improved acute functional outcome and reduced neuronal loss from the striatum. Further studies are required to delineate the mechanism of the effect. (*Stroke*. 1998;29:1917-1923.)

Key Words: behavior, animal ■ hematoma ■ stroke, hemorrhagic ■ rat ■ surgery

Hemorrhagic stroke occurs when a blood vessel or vascular anomaly ruptures, releasing blood into the surrounding brain tissue. Spontaneous intracerebral hemorrhage (ICH) represents one of the most devastating types of stroke,¹ occurring annually in 12 to 35 persons per 100 000 population, and accounting for 8% to 14% of all strokes.^{2,3} Most clinical cases are associated with hypertension, and the most common sites of ICH are striatum, cerebellum, and pons.⁴ The 30-day mortality rate is 43% to 51%, and most survivors are left with a neurological disability.⁵⁻⁷

Current management of ICH includes control of systemic hypertension and treatment or prevention of raised intracranial pressure. While most agree that cerebellar and superficial lobar hematomas should be removed, there is controversy concerning the use of surgery for deep hematomas in the basal ganglia.⁸ Different forms of surgical management, for example, open craniotomy,⁹ stereotactic injection of thrombolytic agents to facilitate clot lysis and removal, or the use of endoscopy,¹⁰ have been described as treatments for ICH. A meta-analysis of randomized clinical studies in 1997 suggested that immediate surgical removal of hematomas might improve outcome in noncomatose patients <60 years of age with a hematoma volume of <50 mL.¹¹ The authors emphasized that the problem needs to be studied in a randomized trial.¹²

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The effects of ICH have been studied experimentally using infusion of autologous blood and implantation of inflatable balloons.¹³⁻¹⁶ To achieve a more reproducible hematoma, Rosenberg and coworkers^{17,18} developed a rat model in which intrastriatal injection of bacterial collagenase was used to disrupt the basal lamina of cerebral capillaries and cause bleeding into brain tissue.

The purpose of this study was to test the hypothesis that aspiration of collagenase-induced hematoma from rat brain is associated with improved neurological outcome. Detailed histopathological assessments were correlated with behavioral tests.

Materials and Methods

Intracerebral Hemorrhage

All experimental procedures were done in accordance with the guidelines of the Canadian Council on Animal Care. Protocols were approved by the local experimental ethics committee. Sixty-six young adult male Sprague-Dawley rats weighing 175 to 250 g were used. Forty-four rats were underwent behavior testing followed by pathological exam 7 weeks after hemorrhage. Fourteen rats were killed 24 hours after ICH for brain-water determinations. Eight rats were used for assessment of streptokinase injection. For induction of

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hemorrhage, each rat was anesthetized with pentobarbital (50 mg/kg IP) and placed in a stereotaxic frame (David Kopf Instruments). Through a hole drilled in the skull, a 30-gauge needle was introduced into the caudate nucleus (3 mm lateral to midline, 0.02 mm anterior to coronal suture, depth 6 mm below the surface of the skull), and 1.4 μ L of saline containing 0.3 U collagenase (Type IV, Sigma Chemical Co) was infused over 7 minutes. After the infusion, the needle was left in the place for 3 minutes and then removed. Physiological parameters were not monitored during the procedure. The bone hole was sealed with bone wax, the scalp wound was sutured, and the animal was placed in a box with free access to food and water. Every second rat with collagenase injection was selected for aspiration of the hematoma. Four hours after collagenase injection, the rat was reanesthetized with pentobarbital (50 mg/kg IP) and again placed in the stereotaxic frame. Using the same stereotaxic coordinates, streptokinase (3 μ L; 1000 U/ μ L, Sigma) was injected by a 27-gauge needle into the hematoma center. One hour later, aspiration was accomplished by application of gentle suction with a syringe attached to a 25-gauge needle placed at the same stereotaxic coordinates. Physiological parameters were not monitored in this experiment. The volume of aspirated blood was measured. Eight rats without ICH were injected with the same quantity of streptokinase in 1 side and an equal volume of saline in the contralateral striatum and were killed 1, 3, 7, or 11 days later for histological assessment.

Behavioral Testing

All testing was done by a single observer without knowledge of the treatment group. Motor behavior was evaluated using 4 tests in each rat 1, 3, 5, 7, 11, 14, 17, 21, and 28 days after collagenase injection. The specific tests included (1) observation of spontaneous ipsilateral circling, graded from 0 (no circling) to 3 (continuous circling); (2) contralateral hindlimb retraction, which measured the ability of the animal to replace the hindlimb after it was displaced laterally by 2 to 3 cm, graded from 0 (immediate replacement) to 3 (replacement after minutes or no replacement); (3) beam walking ability, graded 0 for a rat that readily traverses a 2.4-cm-wide, 80-cm-long beam to 3 for a rat unable to stay on the beam for 10 seconds; and (4) bilateral forepaw grasp, which measures the ability to hold onto a 2-mm-diameter steel rod, graded 0 for a rat with normal forepaw grasping behavior to 3 for a rat unable to grasp with the forepaws. The scores from all 4 tests, which were done over a period of about 15 minutes on each assessment day, were added to give a motor deficit score (maximum possible score, 12).

Skilled forelimb function was also tested using a staircase feeding apparatus.¹⁹ This required pretraining before induction of ICH. Rats had free access to food and water during first 2 days after arrival from the supplier. The rats were housed in pairs in standard plastic boxes with a 12-hour day/night cycle. During the following 7 days, the rats were fed 8 to 15 g/d of standard laboratory chow to reduce their body weight to 85% to 90% of the initial weight. Hunger was the incentive to perform in the testing apparatus. The staircase pretraining was performed twice per day, with a time interval of at least 4 hours between trials. The rat was placed in a clear plastic box with a food-baited staircase on either side. Each staircase had 7 steps, each with a well containing 3 45-mg pellets (P.J. Noyes Co Inc). The number of food pellets reached and eaten in 20 minutes was counted. When a plateau was reached, the top well was no longer baited with pellets, because these can be reached with the tongue. An additional 4 to 6 trials were used to calculate mean pretraining number of pellets eaten from each side. If the side-to-side difference was >4 on the final trials, the side on which rat collected more pellets was designated its "preferred" side. ICH was induced in the dominant brain hemisphere in rats with a preferred side. After pretraining, the rats were allowed free access to food for 2 days before surgery and during the 4 weeks after ICH. Beginning 28 days after ICH, the rats were fed 10 to 12 g/d standard laboratory chow to decrease the body weight to 90% of the free feeding level. They were then evaluated daily in the staircase apparatus for 3 weeks. The top well of the staircase apparatus was not baited. The number of food

pellets eaten in 20 minutes on each side was counted (maximum possible 18 per side).

Histological Examination

Seven weeks after collagenase injection, each rat was reanesthetized and perfused through the heart with 300 mL cold 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline. The brain was removed and stored in the same fixative. Fixed brains were cut coronally through the needle entry site (identifiable on the brain surface), as well as 2 mm anterior and 2 mm posterior to that plane. Brain slices were dehydrated and embedded in paraffin. Sections (5 μ m) were cut, and each 10th section from the rostral to the caudal portion of the residual hematoma cavity was stained with hematoxylin and eosin.

All sections were inspected macroscopically and microscopically to determine anatomical structures involved in the hematoma site. According to the part of striatum involved, hematomas were classified as being medial, lateral, or whole striatum location. Extension of the hematoma into the internal capsule was semiquantitatively graded: 0, no extension; 1, extension in anterior limb; and 2, extension into posterior limb. Extension of the hematoma into the thalamus was similarly graded: 0, no extension; 1, focal calcium deposition and cell loss; and 2, residual cavity.

A "camera lucida" was used to assess the overall brain morphology on the coronal slice with the maximum hematoma diameter. The ipsilateral cortical injection site lesion, hematoma cavity, residual striatum, and ventricle were traced onto a sheet of paper, as were the contralateral striatum and ventricle. Computerized planimetry was used to measure the traced areas. Side-to-side differences were compared. Striatal area loss was calculated as the percentage difference between contralateral and ipsilateral striatum.

Medium-size striatal neurons were quantified at the coronal level of the maximum hematoma diameter as previously described.²⁰ With a square ocular graticule and $\times 250$ ocular magnification (objective magnification $\times 20$), neurons were counted in three fields (each area $400 \times 400 \mu$ m) immediately adjacent to the hematoma site; areas with large blood vessels were avoided. Three anatomically comparable fields in the contralateral caudate nucleus were assessed in the same manner. The difference between the sums from each side was used as an index of relative neuronal depletion in striatal tissue adjacent to the hematoma.

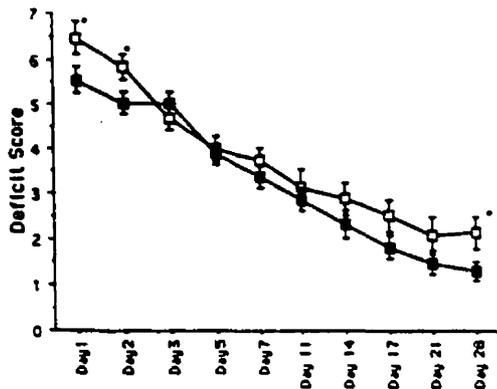
GFAP immunohistochemical labeling was performed on sections at the coronal level of the maximum hematoma diameter. Sections were incubated with 20% goat serum for 30 minutes, then the primary polyclonal GFAP antibody was applied overnight (Dako, dilution 1:400). Secondary biotinylated goat anti-rabbit antibody (1:300) was applied for 1 hour, followed by streptavidin HRP and DAB. Areas with labeled astrocytes were compared between ipsilateral and contralateral side in the cortex and internal capsule and assigned a grade of 0 if the same, 1 if slightly greater, as 2 if much greater on the side of the hematoma. Reactive gliosis extension beside the residual cavity was measured with a calibrated ocular graticule.

Water Content

Fourteen rats were used for this experiment. Six rats had collagenase-induced hemorrhage, and 8 had collagenase-induced hemorrhage followed by aspiration as described above. Twenty-four hours after ICH, the motor-behavior tests were done, then each rat was killed by pentobarbital overdose. The brain was quickly removed and placed on a cooled surface, and the cerebellum and brain stem were removed. The cerebrum was divided into hemispheres, and each hemisphere was coronally cut into 3 parts; the first cut was through the needle entry site and the second through the midpoint of the posterior remnant. Each section was weighed, wrapped in pre-weighed aluminum foil, dried for 3 days in an oven at 110°C, and weighed again. Water content was calculated as the percentage change between wet weight and dry weight.

Data Analysis

All data are presented as mean \pm SEM. Data were analyzed using StatView version 4.1 (Abacus Concepts, Inc). Z-score histogram



Line graph showing motor deficit scores (mean ± SEM) in untreated rats with naturally evolving hematoma (□) and treated rats whose hematoma was aspirated (■). The treated group had significantly better scores (*) on days 1, 2, and 28 ($P < 0.03$; 1-tailed Student's t test).

were used to determine whether the data were distributed normally. For the skilled forelimb test, the mean posthematoma value was calculated for each week (5 trials) for each side separately. The means before and after hematoma were compared. Normally distributed data (behavior, areas of residual cavity, ventricle, striatum, cortical damage, neuronal count, and water content) were analyzed by Student's t test to compare the hematoma and aspiration groups. Correlation coefficient or regression analysis was used to assess the relationship between morphologic features and behavioral outcomes. The Kruskal-Wallis test was used to assess relationship between hematoma location or extension into internal capsule and the functional deficit.

Results

Six rats were excluded before surgery because they refused to eat in the staircase apparatus during the pretraining period. No rats died immediately after surgery. Two rats were euthanized 4 to 5 weeks after ICH because of unexpected weight loss. Histological analysis showed large abscesses in the cortex and striatal region of each rat. One rat was excluded after histological analysis because the bacterial collagenase had been injected into the septal region. For final analysis, there were 18 control rats with naturally evolving hematomas and 17 treated rats with aspirated hematomas. The volume of aspirated blood ranged 20 to 100 μ L. Streptokinase injection into the striatum of rats without ICH was associated with no inflammation, no neuronal changes, and minimal hemorrhage 1 to 11 days later. The changes were similar to those seen on the contralateral side that received injection of saline alone.

Motor deficit scores in the first 4 weeks after ICH are shown in the Figure. The scores were significantly better in the treated group on days 1, 2, and 28 ($P < 0.03$; 1-tailed t test). Results of the skilled forelimb testing are shown in Table 1. The number of food pellets eaten reached a plateau after 8 to 10 trials in the pretraining period. Four rats constantly preferred the right side, 5 preferred the left side. The plateau was reached in 5 trials during post-ICH testing. There were no differences in performance between the groups before ICH. The limb ipsilateral to the hematoma exhibited no loss in performance after ICH. There was a significant

TABLE 1. Skilled Forelimb Testing in Rats With Intracerebral Hematoma

| | Contralateral to Hematoma | | Ipsilateral to Hematoma | |
|--------------------|---------------------------|------------|-------------------------|------------|
| | Pre ICH* | Post ICH† | Pre ICH* | Post ICH† |
| Untreated controls | 11.1 ± 0.6 | 6.0 ± 1.0‡ | 10.7 ± 0.6 | 10.0 ± 1.0 |
| Treated rats | 12.1 ± 0.4 | 6.0 ± 0.9‡ | 10.6 ± 0.6 | 11.2 ± 0.7 |

* Number of pellets eaten daily (mean ± SEM) during pretraining before ICH.
 † Number of pellets eaten daily (mean ± SEM) during last 5 days of testing.
 ‡ $P < 0.05$ vs Pre ICH.

decline in function of the forelimb contralateral to the hematoma, but there was no difference between the treated and untreated rats.

In the treated rats, functional performance on day 1 was not dependent on the location of the hematoma within the striatum, hematoma extension into the thalamus, hematoma extension into the internal capsule (see below), or the volume of blood aspirated. However, the final skilled forelimb performance was dependent to some extent on the hematoma location within the striatum and extension of injury into the thalamus. Rats with medially placed hematomas had the least deficit (Table 2). By regression analysis, in the treated rats there were no significant relationships between the final skilled forelimb performance and the area of cortical damage, the size of the residual striatal cavity, or the volume of blood aspirated.

Localization of the hematomas and anatomical structures damaged by ICH is shown in Table 2. The anterior limb of the

TABLE 2. Brain Structures Damaged by Intracerebral Hematoma and Relationship to Skilled Forelimb Performance

| Structure Damaged* | Untreated Controls (n = 18) | Treated Rats (n = 17)† | No. of Pellets Eaten in Relation to Contralateral Brain Damages |
|-------------------------|-----------------------------|------------------------|---|
| Striatum | | | |
| Medial | 11 | 10 | 7.2 ± 0.8 |
| Lateral | 3 | 3 | 4.7 ± 1.5 |
| Whole | 4 | 4 | 2.9 ± 1.1‡ |
| Internal capsule | | | |
| Damage grade 0 | 1 | 1 | 8.2 ± 3.8 |
| Damage grade 1 | 13 | 12 | 6.1 ± 0.8 |
| Damage grade 2 | 4 | 4 | 4.3 ± 1.3 |
| Thalamus | | | |
| Damage grade 0 | 2 | 1 | 5.6 ± 2.1 |
| Damage grade 1 | 13 | 12 | 6.7 ± 0.7 |
| Damage grade 2 | 3 | 4 | 1.6 ± 0.6 |

* Damage grades are explained in Methods.

† Pattern of brain damage was not different between treated and untreated groups.

‡ Results from skilled forelimb testing in staircase apparatus for combined control and treatment groups. The number of pellets (mean ± SEM) retrieved by the forelimb contralateral to the ICH was subdivided according to the damage location or grade specified. Within the subheadings of striatum, internal capsule, or thalamus, the 3 levels of damage were compared.

§ $P < 0.025$ whole striatum injury vs only medial striatum injury.

|| $P < 0.02$ thalamus grade 2 damage vs thalamus grade 0 damage (Kruskal-Wallis test).

TABLE 3. Relative Size of Brain Structures and Damaged Areas

| Site* | Untreated Controls | Treated Rats |
|--------------------------|--------------------|--------------|
| Hematoma cavity | 1.8±0.3 | 1.7±0.3 |
| Cortical damage | 0.9±0.1 | 1.5±0.2† |
| Striatum, ipsilateral | 4.4±0.5 | 4.0±0.5 |
| Ventricle, ipsilateral | 4.6±0.6 | 3.1±0.4† |
| Striatum, contralateral | 9.7±0.7 | 9.8±0.5 |
| Ventricle, contralateral | 1.9±0.2 | 1.3±0.2 |

* All sizes are reported in mm² (mean±SEM), as measured by planimetry on a single coronal histological section at the level of maximal hematoma diameter.

† $P < 0.05$ vs untreated group (2-tailed *t* test).

internal capsule and portions of the ventroposterior and ventrolateral thalamic nuclei sustained some damage in most rats. The pattern of damage was the same in the 2 groups. The relative sizes of the residual damage are shown in Table 3. The ipsilateral ventricle was less enlarged in the rats treated with aspiration, suggesting that there may have been less striatal atrophy. The cortical lesion at the needle entry site was larger in the treated group, probably as a result of repeated needle insertions.

Striatal tissue surrounding the hematoma in untreated rats exhibited significantly greater neuronal loss than in treated rats (54±8 versus 16±7, $P = 0.0014$). There was no difference in the absolute neuronal count in the contralateral striatum between the 2 groups. Reactive gliosis extended an average of 416±56 μm from the residual cavity in untreated rats and 296±40 μm in treated rats ($P < 0.04$; 1-tailed Student's *t* test). There was no significant difference in cortical or external capsule gliosis between the 2 groups.

Brain-water content 24 hours after collagenase injection is shown in Table 4. Water content was significantly increased in the cerebrum ipsilateral to the hematoma compared to the contralateral side in both animal groups. However, there was no difference in water content between the treated and untreated groups.

TABLE 4. Water Content in Brain Slices 24 Hours After Intracerebral Hemorrhage

| Location | Untreated Controls | Treated Rats |
|---------------|--------------------|--------------|
| Contralateral | | |
| Anterior | 80.6±0.3 | 80.9±0.5 |
| Middle | 79.8±0.3 | 79.5±0.7 |
| Posterior | 79.8±0.4 | 79.7±0.3 |
| Ipsilateral | | |
| Anterior | 81.2±0.3* | 81.1±0.4* |
| Middle | 80.7±0.3* | 80.9±0.4* |
| Posterior | 80.0±0.4 | 79.9±0.3 |

Percent water content calculated by wet weight/dry weight differences, mean±SEM.

* The ipsilateral anterior and middle sections include the hematoma site and had significantly greater water content ($P < 0.05$) than the corresponding contralateral sections.

Discussion

There is considerable controversy regarding the value of surgical therapy over conservative therapy for spontaneous intracerebral hemorrhage. Although many studies have been reported, most are considered inadequate to quantify reliably the risk and benefit of surgical treatment. Two recent independent reviews of the literature with meta-analysis assessment^{11,21} determined that there are only 4 randomized trials of surgical treatment worth considering.^{9,10,22,23} Both groups of authors concluded that there was insufficient information on the safety and efficacy of surgery and that more information was needed from a multicenter randomized trial to determine whether some patients with ICH would benefit from surgery.

Pilot experiments using magnetic resonance imaging before and after hematoma aspiration showed that the central contiguous portion of the hematoma could be removed (M.R. Del Bigio, unpublished data, 1997). Pilot experiments with 10 rats subjected to intrastriatal autologous blood injection of 40 to 100 μL showed that the resulting hematoma was very irregular, with extension along the white tracts; the histological changes adjacent to the hematoma, however, were almost identical to those seen in the collagenase model (H.J. Yan and M.R. Del Bigio, unpublished data, 1997). Because we believed that hematoma removal could be accomplished only in contiguous regions, we chose the collagenase model for this study. We wished to determine whether surgical aspiration of collagenase-induced hematoma could improve the final outcome in rats.

Intracerebral hemorrhage causes brain damage by multiple mechanisms. Direct tissue destruction by the hemorrhagic event and dissection of blood along tissue planes occurs immediately. Damaged cells and axons in the path are unlikely to be saved by any intervention. The space-occupying effect of the hematoma compromises local blood flow in the surrounding tissue. This has been shown by a variety of experimental methods after inflation of balloons or injection of autologous blood in the brain²⁴⁻²⁹ and in a small number of ICH patients by CT scanning combined with xenon inhalation.³⁰ Our preliminary experiments in this model using magnetic resonance perfusion imaging³¹ also indicate that blood flow is reduced in an area much larger than the hematoma itself (J. Peeling and M.R. Del Bigio, unpublished data, 1997). These data suggest that ICH has a penumbral region similar to that adjacent to ischemic brain damage in which blood flow is reduced and neuronal function and survival are compromised.³² Important to note is the observation that deflation of a 50- μL balloon implanted into rat caudate nucleus was associated with recovery of cerebral blood flow.³⁴ This may explain why we observed improved neuronal survival and reduced reactive gliosis in the striatum adjacent to hematomas that had been treated by aspiration.

In this experiment, we observed a significant treatment-related improvement in motor behavior during the first 2 days after ICH. Experiments with temporary space-occupying lesion created by balloon inflation in the caudate nucleus of rats indicate that there is a significant increase in intracranial pressure.^{24,28} Intracerebral pressure in pigs with ICH is reduced after lysis of the clot using tissue plasminogen activator and aspiration.^{13,34} Thus, the improvement we wit-

nessed probably was related to relief of the space-occupying effect of hematoma, decreased intracranial pressure, and possibly increased local blood flow. Another postulated effect of ICH is the release of toxic agents, particularly thrombin, from the clotting blood.^{35,36} Aspiration of blood presumably would reduce the quantity of these agents in the brain. However, we know from our short-term experiments and the observation of residual hemosiderin that not all of the blood can be removed. Without precise information concerning the dose-response curve of these agents, it is impossible to know whether partial removal of the blood reduces their effect. A third consideration is a beneficial effect of the additional 1-hour period of anesthesia. For example, deep anesthesia induced by 5-hour thiopental infusion is associated with reduced acute infarct volume after transient middle cerebral artery occlusion in rats.¹⁷ However, this seems unlikely in the present experiment, because in our previous study rats that were anesthetized repeatedly with pentobarbital for the purposes of early sequential MR imaging showed no benefit.²⁰

The acute motor improvement in this experiment did not appear to be due to reduction of brain edema, which develops in the first few hours and peaks at 24 to 48 hours.²⁰ In contrast, Wagner and coworkers¹³ documented reduced perihematoma edema in the pig ICH model after tissue plasminogen activator-assisted evacuation. The edema associated with autologous blood injection into the brains of rats and pigs has a similar time course.^{29,33} Rosenberg and coworkers³⁸⁻⁴¹ have successfully reduced brain water in rats with collagenase-induced hematomas using a variety of drugs, but outcomes were measured at 24 hours and behavior was not assessed in detail.

Despite our documentation of acute motor improvements, reduced neuronal death in the striatum, and reduced striatal atrophy, surgically treated rats did not exhibit any late benefit with regard to skilled forelimb performance. This is likely a function of the brain structures that were acutely damaged by the hematoma. Most of the hematomas extended into the thalamus and internal capsule. These provide assessment of goal-directed movement abilities.¹⁹ For successful reaching and grasping in the staircase test, the corticospinal tract, the basal ganglia, and the ascending sensory pathway should be intact.^{19,42-44} Axonal damage in the internal capsule and thalamic injury would be expected to affect the outcome of this test, and it is very unlikely that axonal damage would be amenable to aspiration of blood from the striatum. We must consider the possibility that the cortical damage caused by repeated needle insertions was detrimental. It is also worth noting that hematomas involving either the lateral or entire striatum were associated with greater disability than those associated with only medial damage. This has been shown directly in other experiments in which lesions of the lateral striatum produced severe and chronic impairments of movement initiation, forelimb-reaching amplitude, and postural synergism but damage to the medial striatum produced mild or no impairment of forelimb reaching.⁴⁵

We conclude that partial surgical aspiration of collagenase-induced intracerebral hematomas from rat brains improves the acute functional deficit slightly, probably through reduction of the space-occupying effect of the hematoma and

consequent reduction of intracranial pressure. Acutely damaged axons do not benefit by surgical treatment. Late neuronal survival in the striatum surrounding the hematoma was also improved, possibly as a result of improved local cerebral blood flow or removal of potentially toxic blood breakdown products. It appears that intracerebral hematomas are associated with a penumbra similar to that surrounding ischemic brain tissue, in which selective neuronal loss can occur. Further investigations into the value of drug therapy to treat cerebral edema and neuronal ischemia combined with surgical treatment are warranted.

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Editorial Comment

In the article published above, Altumbabic and colleagues induced deep intracerebral hemorrhage in rats by injection of bacterial collagenase. Every second rat was reanesthetized 4 hours later, streptokinase was injected, and the hematoma was aspirated. The groups were compared on "behavioral function," histopathology, and brain edema. Hematoma aspiration resulted in a small improvement in "motor deficit score" 1, 2, and 28 days after hemorrhage, which was determined by assessing a combination of 4 specific tests. On another test of skilled forelimb function, no differences could be detected between groups. Hematoma aspiration reduced neuronal loss and reactive gliosis but not edema. The authors argue that the additional anesthesia time in the clot removal group did not favorably affect outcome, but this remains a possible explanation for the improvements observed, particularly in the absence of measurements of vital signs, including brain temperature.

The experiment was done to address the clinical question of whether early hematoma evacuation improves functional outcome. The model used, however, does not produce clinical

differences of the magnitude observed in humans. None of the rats died from the effects of intracerebral hemorrhage, unlike the clinical situation, in which up to 50% of patients with deep intracerebral hemorrhage die. A 10% to 15% improvement in motor deficit score was observed and was associated with a much more marked 70% reduction in neuronal loss. This highlights the difficulty of using tests of function in rats. Investigators have relied almost exclusively on histopathological endpoints in the study of experimental cerebral ischemia. A 70% reduction in infarct size, if approximately equal to the 70% increase in neuronal survival noted in this study, would be a marked effect.

The pathogenesis of neurological deficit and death is certainly multifactorial and includes direct effects of the hematoma causing direct destruction of brain tissue, destruction by mass effect and brain shift, ischemia, toxic effects of substances released from the blood clot, and secondary induction of edema, brain swelling, increased local pressure, and diffuse intracranial pressure. Broderick et al¹ reported

that continued bleeding or rebleeding also may be a common cause of deterioration and morbidity and mortality. The rat model reproduces some of these features. It stands to reason that removing the clot early would prevent or decrease damage due to some of these mechanisms and therefore have the potential to improve outcome. The conclusions that are usually drawn from the prior clinical trials and other pertinent literature are as follows.²⁻⁴ Cerebellar and cerebral lobar hemorrhages should be removed surgically unless the patient is too well to need surgery or does not need surgery to make a diagnosis or remove the lesion that caused the hemorrhage, or if the chances of functional outcome are nonexistent. The following applies to hemorrhage in the pons, thalamus, and putamen, which are the most common sites for hypertensive hemorrhage and in general to patients with Glasgow Coma Scale scores between 7 and 12 or so who are not either very well or very ill. For patients such as this, with pontine and thalamic hemorrhage, surgery performed with some delay of hours after the ictus may increase the survival rate, but those who do survive are usually severely disabled. The questions to be answered, which apply more to thalamic than pontine hemorrhage, are whether removing the hematoma with less disruption of the brain, such as by a stereotactic method, or doing so sooner after the hemorrhage, will improve outcome. For putamenal hemorrhage, surgery decreases mortality but most of the survivors are disabled. There is more enthusiasm for studying whether earlier or less "invasive" hematoma evacuation will improve outcome.

Animal models are important for investigating the pathogenesis of intracerebral hematoma and the effect of neuro-

protective strategies, but the call for a clinical trial has been made so many times that the decision does not rest on results of more experimental data. Experimental studies are unlikely to be able to answer the question of effect on functional outcome. Answers to clinical questions such as this one, in which randomization is difficult, have been sought by prospectively collecting large numbers of patients.

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Transplantation of fetal brain tissue into the site of intracerebral hemorrhage in rats

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Abstract

The purpose of this study was to evaluate whether transplantation of fetal forebrain tissue into the hematoma site of rats with intrastriatal hemorrhage could improve the final neurological outcome. Nine to twelve days after collagenase-induced intracerebral hemorrhage, day 14 fetal forebrain fragments were transplanted into hematoma site. Quantitative measures of behavioral function were repeatedly evaluated until the rats were killed 10 weeks after grafting. Histology was used to assess the survival of the grafts and overall brain morphology. Surviving grafts were located in the residual cavity at the hematoma site. However, comparison of rats with live transplants to control rats with no transplant, sham transplant, or dead tissue transplant revealed no statistically significant differences in any of the motor tests. © 1998 Elsevier Science Ireland Ltd. All rights reserved

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Spontaneous intracerebral hemorrhage (ICH) remains one of the most devastating types of stroke [8], occurring annually in 12–35 persons per 100 000 population, and accounting for 8–14% of all strokes [13]. Current medical management of ICH includes control of systemic hypertension and treatment of raised intracranial pressure. Currently, there are no clinically effective means for restoring brain function in stroke patients.

Fetal brain tissue transplantation is a method under investigation for promoting brain recovery following experimental ischemic stroke [4]. In two experiments, striatal ischemia induced in rats by occlusion of the middle cerebral artery was followed 2 or 8 weeks later by transplantation of fetal forebrain cells. Behavioral improvement and increased production of GABA was observed as early as 4 weeks later and persisted for up to 1 year [1,15]. This approach has not been studied in models of hemorrhagic stroke involving the basal ganglia. The purpose of this study was to test the effect of transplanted fetal rat forebrain tissue on motor outcome in rats with collagenase-induced striatal hematoma.

All experimental procedures were done in accordance with the guidelines of the Canadian Council on Animal Care. Thirty-four adult male Sprague–Dawley rats (225–275 g) were housed individually in reversed day/night lighting conditions. Under pentobarbital anesthesia (50 mg/kg i.p.), bacterial collagenase (0.3 U of type IV collagenase in 1.4 µl of sterile saline; Sigma, MO) was injected stereotactically to produce intrastriatal hematoma as previously described [2]. Nine to twelve days after hematoma induction the rats were randomly assigned to one of four groups: untreated hematoma ($n = 5$), hematoma followed by transplantation of live tissue ($n = 16$), hematoma followed by transplantation of microwave-killed tissue ($n = 5$), or hematoma followed by sham transplantation (needle insertion only for 30 s) ($n = 5$). Four locally mated female rats, three 14 days postcoital and one 15 days postcoital, were used. They were anesthetized with pentobarbital, the uterus was removed, and embryos were isolated under aseptic conditions. The membranes were stripped and the telencephalic vesicles were pinched off and placed in DMEM culture medium (Gibco BRL). Forebrain tissue fragments were incubated with 18 µM Hoechst 33342 (10 µg/ml bisbenzimidazole trihydrochloride; Sigma) for 1 h at 37°C in the dark as done previously to allow identification in the brain [6]. Tis-

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sue was washed with 0.9% sterile saline and coated with sterile charcoal as a site marker. Two pieces of embryonal forebrain tissue (approximately 0.2 mm diameter) were injected into hematoma site through a glass cannula.

Behavioral testing was done by a single observer without knowledge of the treatment group. Prior to ICH, the rats underwent a 16 trial training session in the use of a staircase feeding apparatus that tests skilled forelimb function [14]. Motor behavior was evaluated using three tests in each rat 1, 3, 5, 7, 14, and 21 days after induction of ICH. The tests included observation of spontaneous circling toward the side of the lesion, contralateral forelimb reaching, and beam walking ability as previously described [2]. A deficit score (maximum 8) was assigned based on these tests. In the 4th week after transplantation, rats were placed on a 6 cm diameter cylinder rotating at 20–25 rpm. Time spent on the cylinder was measured (maximum 3 min). The same day, square pieces of tape (6 × 6 mm) were simultaneously placed on the lateral aspect of both forelimbs and the time necessary to remove the pieces of the tape by mouth was measured [3]. Rats were evaluated daily in the staircase feeding apparatus for 4 weeks beginning the 3rd week after transplantation. The mean number of food pellets eaten in 20 min on each side was counted (maximum possible 21 per side) during the 7th week after transplantation. Spontaneous activity was measured twice in the 8th week after transplantation using an infrared beam activity monitor (65 cm diameter opaque cylinder; Lehigh Valley). Rats were placed in the apparatus for 10 min and number of beam interruptions was registered [9]. To assess lateralizing tendency, rats were suspended by the tail 10 cm above a surface for 30 s. The number of swinging episodes to each side was counted: rotational swinging in one direction greater than 70% of the time is indicative of unilateral neurological deficit [5]. Ten weeks after transplant, apomorphine-induced (0.1 µg/kg apomorphine s.c.) circling was assessed with video recording for 15 min. The preference (if any) was expressed as the percent rotations toward the lesion side. All data were quantified and analyzed by ANOVA with post-hoc Bonferroni–Dunn intergroup comparisons or regression analysis as appropriate. Differences were considered significant at $P < 0.05$.

Rats were killed 10 weeks after transplantation by transcardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffered saline. Brains were embedded in paraffin for histological analysis. Paraffin sections were stained with hematoxylin and eosin or coverslipped unstained for viewing by epifluorescence microscopy.

Three rats were excluded prior the surgery because they refused to eat in the staircase apparatus. Five rats with live transplants were sacrificed or died prior to completion of the behavioral testing.

Histological analysis of surviving rats showed that the hematoma in untreated rats and in those with sham transplants was totally resorbed. Only a collapsed residual cavity with hemosiderin-containing macrophages and reactive

astrocytes remained, similar to that previously described [7]. The ipsilateral striatum was atrophic and the ipsilateral ventricle was enlarged. In rats with microwave-killed transplants, particles of charcoal were seen in or near the residual cavity but the pathological changes did not otherwise differ.

Histologically viable transplants consisting of mature glial tissue and ganglion cells were seen in 8 of 16 rats that had received live grafts. The host/transplant margins were usually apparent because of differences in cell and fiber orientation, however, there were no specific anatomical barriers between the two. Donor cell nuclei exhibited blue fluorescence under ultraviolet excitation. Five transplants were large, from 4 to 8 mm diameter. In seven of eight rats, the transplant extended from the residual hematoma site into the lateral ventricle. Growth of transplanted tissue to the intraventricular foramen was associated with contralateral ventricular enlargement in 4/8 rats. The largest transplants compressed the surrounding brain tissue. There was minimal glial reaction at the transplant host interface.

Comparing rats with successful live transplants to control rats in the other groups, there were no significant differences in any of the behavioral scores, assessed before or after transplantation at any time point (Table 1). There was no relationship between the transplant size and the rats' motor performance. Ventriculomegaly had no statistically significant effect on the rats' motor performance.

Fetal striatal neurons have been shown to survive and integrate when grafted into the striatum, in some cases establishing functional connections with host neurons [17]. Early improvements following transplantation of fetal neurons have been attributed to production of trophic substances and/or neurotransmitters [4]. The fact that we did not see any functional improvement associated with the transplant could have several explanations. It is possible that the behavioral tests used in our study were not able to detect minor improvements. However, with the range of tests performed one would expect to have seen improvement in at least one parameter. Our animals were tested only up to 10 weeks after grafting and we cannot exclude the possibility that a longer survival period might have been associated with improved functional outcome when true neuronal connections became established. However, improvements following ischemic striatal injury have been observed 4 weeks after transplantation [15], therefore, we felt justified in discontinuing our experiment at 10 weeks. The donor cells may not have produced any usable neurotransmitters or trophic agents capable of supporting the host neurons. This is perhaps not surprising when one considers that ICH is associated with considerable mechanical disruption of tissue which, in contrast to selective neuronal death, would not be compensated for by a transplant.

One problem with this experiment was the uncontrolled growth of fetal brain tissue. Large grafts behaved as tumors, compressing the surrounding brain tissue and causing hydrocephalus. Similar complications have been reported in a person who received a fetal mesencephalon transplant

Table 1

Motor behavior testing in rats with intracerebral hemorrhage and brain fragment transplants (A)

| | Untreated control lesion (n = 5) | Sham transplant (n = 5) | Dead transplant (n = 5) | Live transplant (n = 8) |
|---|----------------------------------|-------------------------|-------------------------|-------------------------|
| Deficit score Day 1 post-ICH (pre-transplant) | 5.0 ± 2.1 | 4.7 ± 2.1 | 5.4 ± 1.0 | 3.4 ± 2.1 |
| Deficit score day 21 post-ICH (B) | 1.7 ± 1.3 | 2.1 ± 1.5 | 2.1 ± 1.2 | 1.6 ± 1.5 |
| Contralateral feeding pre-ICH (C) | 11.6 ± 1.9 | 10.8 ± 1.9 | 12.9 ± 1.0 | 10.6 ± 2.2 |
| Contralateral feeding post-ICH (D) | 10.8 ± 1.3 | 6.9 ± 6.9 | 8.1 ± 3.7 | 10.3 ± 3.6 |
| Rotating beam agility (E) | 13.8 ± 11.4 | 5.3 ± 2.4 | 36.5 ± 43.0 | 13.0 ± 8.4 |
| Tape test (F) | -28.2 ± 69.6 | -21.1 ± 86.8 | 38.1 ± 86.2 | 8.3 ± 6.9 |
| Spontaneous activity (G) | 628 ± 170 | 663 ± 196 | 682 ± 190 | 616 ± 165 |
| Apomorphine-induced rotation (H) | 50.6 ± 35.0 | 63.8 ± 25.0 | 63.4 ± 37.6 | 58.8 ± 5.1 |

(A). All data are expressed as mean ± SD. There were no statistically significant differences between any of the groups (ANOVA). Details of behavioral tests appear in the methods; (B), deficit score week 2 post-transplant; (C), number of pellets eaten in staircase apparatus during pretraining period prior to hemorrhage; (D), number of pellets eaten in staircase apparatus; week 7 post-transplant; (E), time (s) on rotating beam until falling; week 4 post-transplant; (F), time (s) from removal of tape from forelimb ipsilateral to ICH until contralateral removed; week 4 post-transplant. A negative value indicates that tape from the affected forelimb was removed first; (G), number of infrared beam interruptions in 15 min; week 8 post-transplant; (H), percent of rotations toward side contralateral to ICH; week 10 post-transplant.

for treatment of Parkinson's disease [10]. Although transplant size did not seem to influence the outcome within the transplant group, we cannot exclude it as an explanation for the lack of benefit.

Borlongan reviewed the literature concerning transplantation of fetal tissue as a method for improving brain recovery in animal models of stroke. At present the results are far from conclusive [4]. Some investigators suggest that transplants are only beneficial in animals cared for in an enriched environment [11,12] which can stimulate brain plasticity and is associated with increases in cortical thickness, protein content, and dendritic branching [18]. Standard laboratory housing conditions provide limited sensorimotor stimulation and opportunity for locomotor activity. However, the frequent testing of our rats is, in itself, a form of environmental enrichment.

We conclude that fetal brain tissue transplantation into the site of collagenase-induced striatal hemorrhage in rats does not appear to be beneficial. This does not exclude the possibility that transplantation of purified neural stem cells or cells engineered to produce specific trophic agents [16] might be helpful in recovery after intracerebral hemorrhage.

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