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A Rabbit Model of Intracerebral Hematoma *

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Summary. The epiphenomena that seem to cause deterioration and death after spontaneous interacerebral hematoma (SICH) might best be studied in an animal model. Therefore, the principles for developing such a model and techniques to study these phenomena were evaluated. Animals will tolerate injection of 3%–5% of their brain volume with a high proportion of clots. Fluorescein can be used to study the blood-brain barrier, and gravimetry to study edema. Others have found that injection of a paraffin/oil mixture can be employed for a control model. Refinement of the fluorescein technique, development of a primate model, and directions for future research are suggested.

Key words: Blood-brain barrier – Brain edema – Cerebral hemorrhage

Introduction

Since it appears that in spontaneous intracerebral hematomas (SICH) it is often not the initial hemorrhage that ultimately leads to morbidity and mortality, but rather secondary phenomena which have not been fully described, efforts to understand these problems may lead to a more effective approach to treatment. Indeed, the initial bleeding episode is apparently relatively acute and soon ceases, presumably due to local clotting and tamponade (Herbstein and Schaumburg 1974). This bleeding is said to cause primarily a splitting apart of the brain rather than tissue destruction as in ischemic strokes, which is the reason patients with hemorrhages who survive may show better recovery than patients with similar initial neurologic examinations who have had infarctions (Fisher 1961). Also only 30%–50% of the patients who die have brain stem hemorrhages due to herniation, suggesting that the rest may be salvageable (Freytag 1968). In fact, a significant number of patients deteriorate clinically (at variable rates), presumably due to these secondary phenomena (Luyendijk 1972; Stehbens 1972).

Several mechanisms have been proposed to explain this deterioration including the effects of local pressure leading to a cycle of ischemia and edema, as well as reactions in the tissue to the presence of blood also leading to edema.

Early evacuation might prevent these phenomena and lead to increased rates of survival and improved neurologic outcome. Indeed, investigations in a number of countries have suggested the efficacy of a more aggressive surgical approach (Kaneko et al. 1983; Kaufman 1982, 1983; Mizukami et al. 1983; Pia et al. 1980).

There has been a complementary effort to develop animal models and to investigate the pathophysiology of SICH and the effect of different medical and surgical therapies (Table 1). Recently, a group of Japanese investigators working simultaneously, but unknown to us, did develop such a model and, as will be discussed, arrived at many similar conclusions (Suzuki and Ebina 1980).

Material and Methods

Model

Rabbits of either sex weighing 2.5–3.0 kg were anesthetized using ketamine, acepromazine maleate, and xylazine. They were positioned in a Kopf stereotaxic head holder with modified oblique ear bars. The coordinates for the left thalamus were determined using a standard atlas (Sawyer et al. 1954). A short midline skin incision was made and the point for a stereotactic approach through the skull determined. A 0.0292 inch drill bit was twisted by hand to perforate the skull. A 2½-inch 22-gauge spinal needle was then introduced to the proper depth and autologous blood slowly injected. From 0.5 to 3.0 cm^3 of autologous blood was injected. Attempts to create a control model were carried out by injecting 1 cm^3 silastic.

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Table 1. Models of intracerebral hematoma

Author	Year	Species (volume)	Site	Technique	Control	Purpose/phenomena
Laurent et al.	1976	rhesus monkey	basal ganglia	infarction, hypercarbia		mechanism
Hamm	1979	rat	superficial	hypertension		etiology
VanderArk and Kahn	1968	monkey	"intracerebral"			role of surgery
Sussman et al.	1974	dog (2 cc)	frontal	freehand injection		pH changes
Kuchivaki et al.	1979	dog (2–4 cc)	frontal or capsule			intracranial pressure
Mohr and Lorenz	1979	cat	variety	stereotaxic injection		volume, extent, intracranial pressure
Ropper and Zervas	1980	rat (0.22–0.29 cc)	caudate	stereotaxic injection		cerbral blood flow
Suzuki and Ebina	1980	dog (2 cc)	internal capsule	stereotaxic injection	+	pathologic changes
Enzmann et al.	1981	dog (1–3 cc)	parietal	freehand injection		sonograms, CTs, pathologic changes
Kaufman	1984	rabbit	thalamus/ basal ganglia	stereotaxic injection	±	blood-brain barrier, edema

Blood-Brain Barrier

The blood-brain barrier was investigated initially with 2.5% Evans blue, and subsequently with 10% fluorescein (Wolman et al. 1981). The animals were killed after various intervals using barbiturates and glutaraldehyde transcardiac perfusion. The brains were removed and placed in glutaraldehyde overnight. The brains were then divided along the needle tract and both portions sectioned in the coronal plane into 5-mm-thick slices. Frozen sections were prepared and examined using a Leitz 50 W epifluorescent microscope with LP 515 and BG 12 barrier filters yielding a wavelength range of 325–410 nm.

Studies of Edema

Gravimetry was used to investigate edema (Fertz et al. 1980; Marmarou et al. 1978, 1982; Nelson et al. 1971; Shigeno et al. 1982). Animals were killed with barbiturates. Their brains were rapidly removed, divided along the injection tract, and cut into 5-mm slices which were immediately placed in kerosene. Specimens were removed from selected anatomic sites, and their specific gravities were determined using a standardized column of graded specific gravities.

Results

Model

A total of 62 animals were studied after injecting 0.5–3.0 cm^3 blood and eight after injecting 1 cm^3 silastic. As noted in Table 2, when 0.5–1.5 cm^3 blood was injected, clots were seen in 35/45 (78%) with 5/45 deaths (11%). However, four of the deaths occurred early in the project and were related to excessive anesthesia. On the other hand, when 2.0–3.0 cm^3 blood was injected, clots were seen in only 8/17 (47%), while 7/17 (41%) animals died. All these deaths occurred within a few hours. Those animals that survived appeared neurologically intact.

The clots were of varying sizes (Fig. 1.). Minor leaking into the ventricles and subarachnoid space occurred with the smaller injections, whereas there was considerable extravasation when 2.0 cm^3 or more was injected.

Table 2. Results of injections of autologous blood

Volume (cc)	Total	With clots			Without clots	
		no.	(%)	(no. died)	no.	(no. died)
0.5	5	4	(80)	(3)[a]	1	
0.75	7	5	(71)	(1)[a]	2	
1.0	31	24	(77)		7	
1.5	2	2	(100)		0	
2.0	9	3	(33)	(1)	6	
2.5	6	3	(50)	(1)	3	(3)
3.0	2	2	(100)	(2)	0	
	62	43			19	

[a] Deaths early in study, probably related to anesthesia

Attempts to use silastic to create a control model were unsuccessful because, when mixed at a high viscosity, it could not be injected through the relatively small needle and, when thinned, it tracked back up around the needle. With more experiments, it might still be possible to develop this control model.

Blood-Brain Barrier

Initial to study the blood brain barrier with Evans blue resulted in staining in only 1/9 animals. However, minor alterations of the blood-brain barrier which may not be visualized with Evans blue, can be detected with fluorescein (see Discussion) (Wolman et al. 1981). Therefore, different volumes of 10% fluorescein were injected at a variety of intervals before killing. It was found that 1.5 cm^3 of 10% fluorescein injected 5 h before killing gave the best results, and 18 animals were studied with this protocol. Twelve of the 18 animals had clots. These animals were killed after 1–7

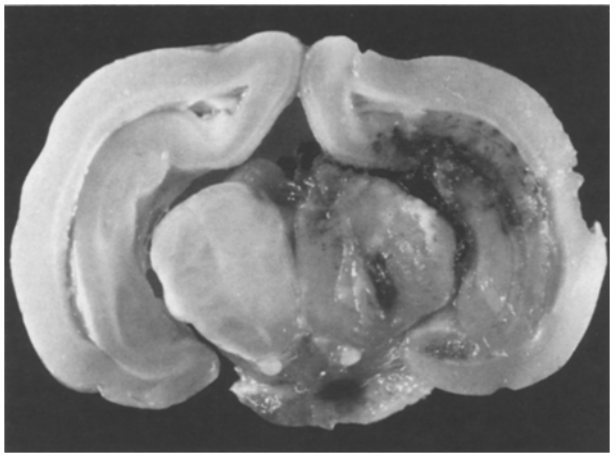

Fig. 1. Rabbit brain divided along needle tract demonstrating thalamic clot as well as some secondary leakage and possibly injury to more distant brain

days. Fluorescence was present around the clots in all ten of the animals who had sections prepared. However, the tissue fragmented during the preparation of slides, apparently due to alteration in its physical characteristics by the clot and associated edema, and this prevented measurements to quantify the results. Refinement of this technique is needed.

Edema

Although preliminary results in two animals suggested that gravimetry can be used to determine specific gravity, it is clear that standards will have to be established and a large number of normal and experimental animals studied.

Discussion

A standardized model of intracerebral hematoma would be extremely valuable, for it would facilitate the study of the pathophysiology of intracerebral hematoma and the development of improved therapy.

With the aid of atlases and stereotaxic equipment, it is possible to place blood precisely into the deep gray structures of animal brains. The volume of blood which can be injected with a high proportion of clots and a low mortality is in the range of $1-1.5\ cm^3$ or $3\%-5\%$ of the normal rabbit brain of 30 g. This is also the approximate percentage used in dogs by Suzuki and Ebani (1980). This volume is equivalent to a clot of approximately $50\ cm^3$ in the human. The clots were of irregular shape and their size was not always predictable, as has been noted in other models. Larger injections resulted in a lower proportion of clots, apparently because blood is forced up the track of the needle or into the ventricles, as well as a higher mortality rate. We expect use of an animal with a larger brain, such as a primate, would permit injection of a larger volume of blood with less problems with blood being lost into the ventricles or up the needle track.

We had hoped to study disruption of the blood-brain barrier by the use of dyes and edema by the use of gravimetry, and to compare changes caused by blood with changes caused by an inert substance, silastic. The failure of the first dye used, Evans blue, to indicate changes in the blood brain barrier has just been explained (Wolman et al. 1981). Evans blue is bound to albumin and therefore acts as a "macromolecular" dye, apparently able to pass through the blood-brain barrier only if it is severely disrupted. On the other hand, fluorescein does not bind significantly to protein and therefore can pass through a blood-brain barrier that is less severely disrupted. It can therefore act as a more sensitive marker of injury to the blood-brain barrier. Other investigators have had similar unsatisfactory experiences using Evans blue (Crockard et al. 1976). Although technical problems prevented application of this technique for quantitative evaluation, it should be possible to overcome these difficulties.

Edema, of course, is the result of the cumulative entrance and exit of fluid into tissue over a prolonged period and must be studied separately from changes in the blood-brain barrier at a given point in time. Gravimetry should be a simple technique to evaluate edema.

Fortuitously, a study with many similarities has been carried out by Suzuki and Ebina (1980). They injected $2\ cm^3$ autologous blood stereotaxically into the internal capsule of 25 dogs. The animals were killed at 0.5, 3, 6, 12, 18, 24, 30, 36, and 48 h until one adequate specimen was obtained for each time. Of note, as in our study, the extent of the clots was variable. A similar series of control animals was prepared using injections of $2\ cm^3$ of a 50% fixative paraffin/50% olive oil mixture. Histological specimens were stained with hematoxylin-eosin (HE) and Elastica Masson. Dogs who had an injection of blood developed a progressive status spongiosus and then rims of perivascular bleeding and necrosis. This process was much more marked than in those animals injected with the paraffin/oil mixture and was attributed to the infiltration of plasma components into the tissue. Thus, this study describes a technique for producing a control model and has proven that there are properties of blood which make it particularly irritating to brain when compared to inert substances. The authors therefore concluded that early (6 h) evacuation of hematomas would minimize these changes and that even removal later on would reduce the changes.

A study by Enzman et al. (1981) complements this work. These authors injected 1 – 3 cm^3 blood into the parietal lobe of 12 dogs and followed the animals for up to 21 days with sonograms, CT scans, and ultimately with neuropathologic examinations (HE, reticulin, hematoxylin von Gieson, and Masson trichrome stains). They divided the pathologic evolution of the lesions into four stages: acute (days 1 – 3), subacute (days 4 – 8), capsule (days 9 – 13), and organization (days 13 and after). They also concluded that there is a reaction to the hematoma involving edema and cerebritis which maximized histologically in the subacute stage. Contrast enhancement on CT was seen around the clot initially in the subacute stage but continued to occur after long periods and indicated breakdown of the blood-brain barrier and then neovascularity.

The principles for further refinement of a model of SICH and of a control model have been established. It would now seem appropriate to apply these principles to develop a model in the primate because the reactions of the brain to blood would be most similar to man and because a larger volume of blood could be injected, making the possibility of testing surgical therapy more technically feasible. A multitude of new in vitro and in vivo diagnostic techniques, including autoradiography, computerized tomography, positron emission tomography, and nuclear magnetic resonance should provide much additional information on the pathophysiology of SICH and the results of medical and surgical treatment. Again, because of the limitations in spatial resolution of many of these techniques, an animal with a larger brain should be employed.

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