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Experimental animal models of intracerebral hemorrhage

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Experimental intracerebral hemorrhage (ICH) has been studied in several models and species, including the rat, rabbit, cat, dog, and primate [1]. We recently developed a porcine lobar ICH model to examine ICH pathophysiology, pathochemistry, and surgical clot evacuation [2]. In all these models, hematomas are produced by infusing autologous blood into specific brain regions. A model introduced by Rosenberg and his colleagues [3,4] employs a local injection of bacterial collagenase into the basal ganglia, which dissolves the extracellular matrix, leading to an intracerebral bleed. Overall, these models have contributed significantly to our knowledge of ICH-induced injury, including the roles of mass effect and elevated intracranial pressure, alterations in blood flow and metabolism, and the impact of specific blood components on brain edema formation and blood–brain barrier (BBB) disruption. These models are also providing details of biochemical and molecular events, and they enable testing of potential therapies, both pharmacologic and surgical. In our review, we first describe the brain pathologic responses to ICH in animal models. We then describe in more detail findings that have been obtained with individual species and models. A comprehensive review of experimental ICH models was published by Kaufman and Schochet [1] in 1992.

Brain pathologic responses to intracerebral hemorrhage in animal models

In general, the brain pathologic responses to an ICH are comparable in human beings and experimental animal models [5–14]. Three stages of sequential change that occur around an ICH were originally defined by Spatz [15] and include initial deformation, followed by edema and necrosis, and finally clot absorption and scar or cavity formation. In the rat, regions of pallor and spongiform change develop adjacent to clots within 2 hours [8]. By 6 to 15 hours, disrupted myelinated nerve fibers and degeneration bulbs are present along with increasing swelling of the corona radiata as edema fluid continues to accumulate. At 24 hours, this white matter edema is more marked and extensive. By 48 hours, the hematoma is surrounded by edema, vacuolation, and acellular plasma accumulations [6], and astrocytic swelling is present adjacent to and distant from the hematoma [8].

In our porcine white matter ICH model, we observe marked, rapidly developing (by 1 hour)

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perihematomal edema, which physically alters the appearance of the tissue [2]. This edematous white matter is "translucent" on frozen brain sections because of its high water content, which can exceed 10% [2,16]. This early edema is seen as perihematomal hyperintensity on T2-weighted MRI at 2 hours after ICH [17], a finding also observed on MRI in ICH patients [18]. Edema volumes increase by 50% over the first 24 hours after ICH because of delayed BBB opening [2,10,16]. This vasogenic edema that is evidenced by BBB permeability to Evans blue–tagged albumin develops with a delay of 8 to 24 hours [2,10,19,20]. On T2-weighted imaging, similar hyperintensities surrounding hematomas and extending along posterior white matter fiber tracts are also observed in the collagenase ICH model [21]. By 3 days, in the porcine lobar ICH model, we observe decreased Luxol fast blue staining in edematous white matter suggestive of myelin injury and markedly increased glial fibrillary acidic protein immunoreactivity indicative of reactive astrocytosis [10,13]. Astrocytosis after ICH may be beneficial by sealing off normal brain from injured tissue [22], and it may also provide trophic substances to damaged tissue and contribute to BBB restoration [23,24]. Conversely, the resulting glial scar may serve as a mechanical barrier to regeneration [23]. Although hematoma volumes remain unchanged and neovascularization is present in the porcine model at 7 days, continued hematoma resolution and glial scar and cyst formation are similar to literature descriptions by 2 weeks [8]. We have observed similar brain pathologic responses in porcine brains in which plasma alone is infused [12]. These results suggest that in addition to the blood's red cell component, intraparenchymal accumulation of plasma proteins plays an important role in ICH-induced brain injury [10,12,25].

Several workers, including our group [11], have reported evidence of DNA fragmentation in ICH models using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining. Matsushita et al [26] localized TUNEL staining to neurons and astrocytes within and outside the clot at 24 hours by double-labeling techniques. Hickenbottom et al [27] demonstrated TUNEL staining that colocalized to cells containing the activated proinflammatory transcription factor nuclear factor-κB. Recent characterization and quantitation of intact, injured, and necrotic cells using hematoxylin–eosin and TUNEL staining in a rabbit lobar ICH model demonstrated large numbers of apoptotic cells within the hematoma [28]. In this model, perihematomal regions at 24 hours contained large numbers of morphologically intact cells and swollen cells that may be reversibly injured.

Several reports have carefully examined the time course of inflammation and cell death after infusions of whole blood into the rat striatum [29,30]. TUNEL-positive dying cells were maximal at 72 hours and persisted until 4 weeks. At 24 hours after ICH, degenerating erythrocytes and fragmented nuclear debris are present in damaged perihematomal tissue. In intact tissue, neutrophils adhered to vessel walls or were present within capillaries and small veins. Maximal numbers of neutrophils were present at 48 hours, and they were essentially gone by 72 hours. Small CD8a immunoreactive cells with minimal cytoplasm and round nuclei believed to be natural killer lymphocytes became apparent at 48 hours and persisted until 1 week. Lectin labeled microglia were evident beginning at 4 hours, were maximal at 48 to 72 hours, and persisted until 4 weeks. In comparison with saline-infused brains, at 48 hours, significantly more inflammatory and dying cells were present after whole blood versus saline injection. The size of the hematoma seemed to be constant for at least 72 hours and perhaps for 1 week, but by 4 weeks, the hematoma was considerably reduced in size.

Similar findings have been reported by Gong et al [29] of a perihematomal inflammatory response after ICH that is characterized by infiltration of neutrophils and macrophages and activation of microglia. These infiltrating leukocytes may be important contributors to injury because they may release vasoactive and cytotoxic mediators. Further support for leukocyte infiltration in tissue injury after ICH is the finding that depletion of leukocytes and platelets by whole-body irradiation in a rat ICH model protects against edema formation [31].

Intracerebral blood infusion intracerebral hemorrhage models

Intraparenchymal infusion (or injection) of autologous blood is a straightforward and effective technique to produce an intracerebral hematoma. Although this method does not specifically reproduce the bleeding event of spontaneous ICH in human beings (ie, punctate blood vessel rupture), it is controllable and reproducible. One disadvantage of blood infusion models is the potential for ventricular rupture as well as for backflow of infused blood along the needle track [32,33]. Such

events can lead to intraventricular and/or subarachnoid leakage of blood. This problem has been addressed in the double-injection ICH model in the rat, in which a small amount of blood is initially infused into the caudate at a slow rate followed by a 7-minute wait to allow the blood to clot along the needle track [32]. The remaining blood is then infused, thereby generating reproducible hematoma volumes without subarachnoid blood accumulation. This model was employed by Hickenbottom et al [27] in their study of NF-κB activation after ICH.

Rats

Rats have been the most frequently used species for ICH studies, with the basal ganglia being the most frequently injected site. In the mid-1980s, several studies examined the relation between mass effect and intracranial pressure and showed a positive correlation between hematoma volume and ICP elevation. Perihematomal tissue was also subject to scrutiny in these studies, and it was suggested that locally reduced blood flow in tissue immediately surrounding the hematoma was responsible for secondary damage [34–39], although recovery of flow and hyperemia were noted by Ropper and Zervas [40].

More recently, in their studies of the pathophysiology of perihematomal edema, the University of Michigan ICH group reported that although some degree of ischemia occurs in the early minutes to a few hours after ICH in rats, the ischemia is not severe and is not the basis for perihematomal edema development [33]. In fact, in the rat and porcine ICH models, hyperemia has been observed [17,38]. Importantly, specific blood components are now known to be essential for ICH-induced perihematomal edema. Xi et al [41] showed that unheparinized blood infusions produced significantly greater edema than heparinized blood infusions. Furthermore, thrombin alone can produce perihematomal edema comparable to that of whole blood [42]. Lysed autologous erythrocytes produce marked edema 24 hours after infusion [42] (and also caused severe brain edema and death in the porcine ICH model [25]). This result was mimicked by hemoglobin infusion, but packed erythrocytes do not produce marked edema [42]. Packed red blood cells do produce a marked increase in brain water content by 3 days after infusion, suggesting a delayed, non-thrombin-mediated, edematogenic component of ICH [42]. Further studies by this group have demonstrated that activation of complement and the formation of membrane attack complex contribute to brain edema formation after ICH [43,44]. *N*-acetylheparin, which inhibits complement activation, diminished perihematomal brain edema [45]. Thus, the complement system may serve as a possible target for the treatment of brain injury after ICH [43–45]. Finally, in a cortical ICH model, extravasated whole blood caused a greater degree of cell death and inflammation than ischemic lesions of similar size [46].

Cats

The relation between size and location of an intracerebral hematoma induced by autologous blood injection in cats was studied by Mohr and Lorenz [47]. Size and location of the clot were found to be important in determining functional deficits and ICP elevations. Kobari et al [48] suggested that increased ICP seemed to be the main cause of cerebral blood volume and cerebral blood flow (CBF) reduction shortly after experimental basal ganglia hematoma induction. Using this same model, Dujovny et al [49] found that when urokinase was injected along with blood, neurologic deficits resolved as internal capsule hematomas disappeared.

Rabbits

Kaufman et al [50] stereotactically injected autologous blood into the thalamus in rabbits. They found that these animals would tolerate clots with a volume of 3% to 5% of their brain volume, which approximates a 50-cm^3 clot in human beings. Narayan et al [51] tested urokinase in a rabbit model for safety and efficacy in lysing intracranial hematomas. Overall, clot lysis was demonstrated in 86% of the urokinase-treated animals and in only 23% of the 13 controls with injections of saline into the clot. There was no histologic evidence of increased damage or inflammation noted on careful light microscopic examination between these animals and an additional 22 rabbits treated with urokinase or saline 24 hours after clot injection. The authors concluded that urokinase may be safely and effectively employed for the lysis of intracranial hematomas in this animal model and that a delay in therapy of up to 24 hours does not significantly compromise its efficacy. Gustafsson et al [52] performed MRI studies after injection of autologous blood in the brain parenchyma and subarachnoid space in rabbits. The authors found that susceptibility-weighted

gradient-echo imaging at 1.5 T is highly sensitive to hyperacute hemorrhage in the brain parenchyma as well as to subarachnoid and intraventricular hemorrhage. Qureshi et al [28] recently described a rabbit lobar ICH model in which autologous blood is injected into the deep frontal white matter under arterial pressure. As described in the previous section on the brain's pathologic response to ICH, these workers used this model to investigate the degree of neuronal injury within and outside the hematoma at 24 hours.

Dogs

Canine models have been extensively used for experimental ICH. In 1975, Steiner et al [53] studied the tolerance of blood injection in the different compartments of the dog's brain. The lethal volume of a bleed was specific for each ICH site. The assumption that death might be a random event was discarded, and the failure of vital functions was considered to be the result of elevated intracranial pressure.

The evolution of ICH was investigated in a canine model by high-resolution sonography, CT, and neuropathologic examination in 12 dogs subjected to an experimental parietal lobe hematoma [6]. The sequence of image changes on CT and sonography in this experimental model closely resembled the findings seen after diagnosis of ICH in patients. In MRI studies in a canine ICH model, Weingarten et al [54] proposed a multifactorial hypothesis to explain the differences in intensity between venous, arterial, and intraventricular blood, and they recommended gradient-echo sequences to identify hemorrhagic lesions in the brain.

Takasugi et al [9] correlated histologic changes with CT appearance of internal capsule hematomas in dogs. The authors identified three distinct stages on the basis of histology and CT. In the acute stage (<5 days), on CT, homogeneous high density was present at the periphery of the hematoma, whereas, histologically, a necrotic layer of tissue existed at the boundary of the clot. In the subacute stage (5–14 days), perihematomal density was decreased with ring enhancement after contrast injection and corresponded to the appearance of immature connective tissue with argentophil fibers. In the chronic stage (>15 days), contraction of the enhancing ring was noted and corresponded to the development of mature connective tissue with collagen fibers.

Using a mongrel canine ICH model, Qureshi et al [55] determined the effect of massive ICH on regional CBF (rCBF) and metabolism, testing the hypothesis that there is persistent perihematomal ischemia after ICH. Despite prominent increments in intracranial pressure (ICP) and mean arterial pressure (MAP) indicative of a significant Cushing response after hematoma induction, the authors found no evidence to support the presence of an ischemic penumbra within the first 5 hours after hemorrhage.

Therapeutic approaches for ICH have been studied in the dog by Qureshi et al [56]. Comparing the efficacy of mannitol and hypertonic saline solution at concentrations of 3% and 23.4%, the authors found that hypertonic saline at the 3% and 23.4% concentrations was as effective as mannitol in controlling intracranial hypertension and that hypertonic saline at a 3% concentration might have a longer lasting effect than the other two agents. None of the three solutions affected rCBF or cerebral metabolism. In their study of the pharmacologic reduction of MAP using the same model, Qureshi et al [57] found that reducing MAP with intravenous labetalol within the normal autoregulatory curve of cerebral perfusion pressure (CPP) had no adverse effects on ICP or rCBF in regions around or distant to the hematoma. They concluded that MAP reduction within autoregulation limits in the acute period after ICH is safe.

Monkeys

Bullock et al [58] studied the effects of acute ICH on rCBF by injection of autologous arterial blood in the caudate nucleus in Vervet monkeys. ICP elevations were noted briefly after blood injection and persisted throughout the experiment. CBF was significantly reduced in all regions of the brain for 1 hour after the ictus. The lowest rCBF values were found in the periphery of the hematoma and were below the ischemic threshold for 90 minutes after the hemorrhage. Macaque monkeys were utilized by Segal et al [59] to evaluate basal ganglia hematoma evacuation after urokinase injection. Urokinase was noted to promote absorption, which correlated with improvement on the clinical examination.

Pigs

As described in the introductory and brain pathology sections, our group has developed a white matter ICH model in pigs [2]. The advantages of the pig as an ICH model include its large gyrated brain and large amount of hemispheric white matter, relatively low cost, and

non–companion animal status. The large brain in this species enables hematoma volumes up to approximately 3 cm^3 to be produced by slowly (10–15 minutes) infusing autologous arterial blood through a plastic catheter into the frontal white matter. Indeed, the large hematomas that can be generated make this model useful to investigate neurosurgical clot evacuation [10,20,60]. This lobar ICH model has clinical relevance for several reasons: (1) the frequency of bleeds into white matter and the basal ganglia are similar [61], (2) lobar ICH is the most frequent hemorrhage site in the young [62], and (3) white matter damage is an important contributor to long-term morbidity after ICH [63,64]. Because white matter is more vulnerable to vasogenic edema development than gray matter [65], this model is especially applicable for studying edema-induced injury.

We have used this model to investigate ICH pathophysiology and pathochemistry [2,10,66–68], edema development [2,10,16,17], the role of blood components [2,12,69], and metabolism [19] as well as to evaluate pharmacologic and surgical treatment after tissue plasminogen activator (tPA)–induced clot lysis [10,13,20,68,70]. As described previously, studies in this model demonstrated the important role of clot formation, retraction, and plasma protein accumulation in perihematomal edema development [2,10–12,25, 41,69]. This conclusion is supported by findings in patients who developed ICH but failed to develop significant edema after thrombolytic treatment despite large intracerebral masses [71,72]. Although red blood cells are responsible for most of the hematoma's mass effect, infusions of packed red cells alone also fail to generate perihematomal edema, and as described previously, nonclotting blood also produces minimal perihematomal edema in rat and porcine models [10,11,25,41,69]. These results support the conclusion that (especially) the early and rapid perihematomal edema that follows ICH does not result from mass effect and possibly reduced perfusion induced by the hematoma.

This model has been especially useful for clot evacuation studies. We have demonstrated that early (3.5 hours) clot aspiration after tPA-induced lysis markedly reduced (by >70%) clot volume and perihematomal edema and protected the BBB at 24 hours after ICH [20]. This reduction in clot volume achieved with tPA liquefaction of the clot was significantly greater than the 37% reduction obtained by mechanical aspiration without tPA. Similarly, Altumbabic et al [73] reported that early (4 hours) clot aspiration after streptokinase treatment with urokinase instillation after collagenase-induced ICH in the rat significantly improved functional outcome and reduced neuronal loss in the striatum. Finally, in an ongoing surgical clot removal study, we recently found that the Possis AngioJet rheolytic thrombectomy catheter (Possis Medical, Inc., Minneapolis, MN, USA) was effective for rapidly removing intracerebral hematomas [60]. This device produced an average 61% decrease in clot volumes in approximately 30 seconds. In contrast, tPA treatment required 45 minutes after the initial CT scan to instill tPA into the clot, wait for clot lysis, and carry out repeated attempts to aspirate clots. AngioJet removal of intracerebral clots did not worsen perihematomal edema versus tPA or control. Our preliminary findings suggest that the AngioJet catheter is as effective as tPA and exceedingly faster for treating ICH.

In addition to the comparability of edema development and brain pathologic response between our porcine model and human ICH, a similar marked reduction in metabolic rate is present in perihematomal tissue in experimental and clinical ICH. In our model, we observed that the energy state in white and gray matter adjacent to the clot was remarkably well-maintained and that glycogen and glucose concentrations progressively increased, suggesting a reduction in metabolism without ischemia during the early hours after ICH [19]. Interestingly, Zazulia et al [74] recently reported a marked reduction in the metabolic rate for oxygen in the perihematomal region in acute ICH patients studied 5 to 22 hours after hemorrhage onset. Furthermore, their findings demonstrated that despite the hypoperfusion observed in these patients, a reduced oxygen extraction fraction was present rather than the increased oxygen extraction fraction that occurs in ischemia. These findings in our porcine ICH model are in complete agreement with the reduced metabolism seen in human ICH. This result provides further support for the usefulness of experimental animal ICH models to provide understanding of the pathophysiologic and biochemical alterations induced in the brain by intracerebral bleeds.

A collagenase model of ICH in pigs has been reported recently by Mun-Bryce et al [75]. Their electrophysiologic findings in this ICH model are described in the following section.

Bacterial collagenase model

This model introduced by Rosenberg and his colleagues [3,4,76] utilizes the local injection of

bacterial collagenase into the basal ganglia to induce an intracerebral bleed. Collagenases are proteolytic enzymes present intracellularly in an inactive form. They are secreted at sites of inflammation by mononuclear cells and some tumor cells. Collagen, the substrate for this enzyme, is known to be present in the basal lamina of cerebral blood vessels. Collagenase dissolves the extracellular matrix around capillaries and opens the BBB. This results in active intraparenchymal bleeding that models spontaneous ICH in human beings. These animal models develop reproducible hemorrhages with volumes that correlate well with the amount of collagenase injected [3,4,77]. The advantages of the method are that the hemorrhage is simple to produce and it is spontaneous without significant blood leakage along the needle track. A disadvantage of this model is that bacterial collagenase introduces a significant inflammatory reaction [30,78,79] more intense than that observed in experimental ICH models employing blood infusion as well as in human ICH [6,14]. In addition, the model differs from the punctate vessel lesion that produces ICH in human beings, because collagenase dissolves the extracellular matrix around capillaries to produce hemorrhage. It should be noted that the apoptotic changes described by Matsushita et al [26] in the collagenase model were believed not to be caused by neurotoxicity of the injected collagenase, because collagenase at similar doses did not induce cell death in primary neuronal cultures. In addition, the neuropathologic alterations in the striatum (ie, TUNEL-positive cells and DNA laddering) with the collagenase model were also present in a blood infusion ICH model.

Nuclear magnetic resonance spectroscopy was performed by Mun-Bryce et al [76] after collagenase-induced ICH in rats. They correlated lactate increases with brain water content and a dissociation of lactate and brain pH in perihematomal regions with vasogenic edema development. The same group of investigators obtained serial MRI scans to study the MRI changes at different times after induction of ICH and correlated them with histologic changes in the same time frame. They were able to characterize the evolution of paramagnetic effects of blood on MRI [21]. These results were reproduced more recently by Del Bigio et al [78,79], who also theorized that an intense neutrophilic response to the induced lesion may contribute to neuronal injury at the periphery of the hematoma.

Mun-Bryce et al [75] recently reported following ICH induced by collagenase injection into the primary sensory cortex in the pig, somatic evoked potential (SEP) elicited by electric stimulation of the contralateral snout, and changes in DC-coupled potential monitored in the somatosensory region. The SEP decreased in amplitude within minutes of the intracerebral injection, and its short-latency component was abolished within the first hour without any sign of recovery. As the SEP amplitude began decreasing, recurring episodes of cortical-spreading depression were noted (by 20 minutes). The timing of spreading depressions in the somatosensory cortex was consistent with their initial generation at multiple sites adjacent to the lesion core and propagation into perihematomal brain. The frequency of these depressions near the injection site decreased with time, shifting to more distant brain regions. Overall, these interesting findings demonstrate that ICH leads to reduced SEP amplitude and spontaneous episodes of spreading depression, which are likely caused by the loss of ionic homeostasis.

Ischemia-reperfusion hemorrhage model

In 1976, Laurent et al [80] described an interesting ICH model in Rhesus monkeys in which hematomas were induced during the vasoproliferative stages of maturing infarction. In this model, MAP elevation at 5 days induced by cerebral vasoconstriction after permanent middle cerebral artery occlusion caused hemorrhagic infarct conversion, whereas cerebral vasodilation caused intracerebral hematoma formation. No further studies have been reported with this model.

Summary

Experimental animal ICH models are able to reproduce the overall important pathophysiologic events documented in human ICH, including edema development, markedly reduced metabolism, and tissue pathologic responses. Thus, ICH models serve as an important tool for new understanding of the mechanisms underlying brain injury after an intracerebral bleed. Currently, ongoing studies in several laboratories using these models investigating secondary inflammatory responses as well as intracellular signaling and molecular events are expected to provide therapeutic targets for treating ICH. Future studies should also be directed at one aspect of ICH modeling that has received little attention—potential differences in the hemostatic systems and physical and

biochemical properties of clots in animals that might make their susceptibility to aspiration and/or fibrinolytic drugs and rates of rehemorrhage different than in human beings. Also, future efforts should be directed toward the development of a model that mimics the pathophysiologic processes that lead to spontaneous ICH, progression of hemorrhage, and the recurrence of bleeding in human beings. This model would not only provide better understanding of the dynamic events leading to ICH and tissue injury but should also lead to the development of highly effective pharmacologic and surgical treatments.

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