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Rodent Models of Intracerebral Hemorrhage

Crystal L. MacLellan, PhD; Gergely Silasi, MSc; Angela M. Auriat, BSc; Frederick Colbourne, PhD

Abstract—The collagenase and whole blood intracerebral hemorrhage (ICH) models are widely used to identify mechanisms of injury and to evaluate treatments. Despite preclinical successes, to date, no treatment tested in phase III clinical trials has benefited ICH patients. These failures call into question the predictive value of current ICH models. By highlighting differences between these common rodent models of ICH, we sought to help investigators choose the more appropriate model for their study and to encourage the use of both whenever possible. For instance, we previously reported substantial differences in the bleeding profile, progression of cell death, and functional outcome between these models. These and other differences influence the efficacy and mechanisms of action of various treatment modalities. Thus, in this review, we also summarize neuroprotective and rehabilitation findings in each model. We conclude that differences between ICH models along with our current inability to identify the more clinically predictive model necessitate that preclinical assessments should normally be done in both. Such an approach, coupled with better assessment practices, will likely improve chances of future clinical success. (Stroke. 2010;41[suppl 1]:S95-S98.)

Key Words: intracerebral hemorrhage ■ rodent model ■ neuroprotection ■ rehabilitation

Intracerebral hemorrhage (ICH) is a devastating stroke with ~50% mortality by 1 month, whereas most survivors remain disabled.1,2 Animal models of ICH have significantly advanced our understanding of pathophysiology, thereby identifying numerous therapeutic targets.3,4 Despite the use of these models, especially in rodents, and the efficacy reported in them,4 no treatments have been proven to help ICH patients. Notably, recent clinical failures call into question whether these models accurately reflect human ICH and whether they will be useful in successfully translating treatments from the laboratory. Similar concerns have been raised for ischemic stroke.

As with ischemia, no model perfectly reflects the complexity and heterogeneity of ICH in humans. Accordingly, preclinical studies should use several models to better understand pathophysiology and to predict clinical efficacy. This article briefly discusses the 2 most widely used rodent models of ICH. Although these can be compared on many facets, we focus on bleeding profile, progression of cell death, and changes in neuroplasticity, which are all of utmost importance to final outcome. Furthermore, we highlight how these model differences impact hypothermic neuroprotection and the effects of rehabilitation.

Animal Models of ICH

Animal models of primary ICH have been developed using pigs, rabbits, dogs, cats, and rodents. The bleed is usually created in the striatum, but other regions have been targeted. Experiments with inert substances (eg, balloon inflation) and blood components (eg, thrombin), to evaluate their contribution to ICH outcome are widespread. However, the most commonly used models involve intraparenchymal infusion of either autologous blood5 or bacterial collagenase,6 usually into rodents. The enzymatic action of collagenase, which disrupts the basal lamina of blood vessels, causes blood to leak into the surrounding tissue. Although both models recreate the fundamentals of human ICH with good face validity, they differ in ways that influence outcome.

Outcome Determinants

Hematoma Size and Location

The size and location of the hematoma are primary factors determining outcome in humans7 and rodents.8 With regard to hematoma size, it was traditionally assumed to result from a single large bleed; however, this is not normally the case, and many patients also undergo continued bleeding or rebleeding.9 These aspects of human ICH are not mimicked in the blood injection model. Thus, the collagenase model is more appropriate for studying hematoma expansion.

As noted, one can easily vary the primary location of injury, but sometimes it is difficult to control lesion shape and spread in these models. For instance, the whole blood model results in a narrower slit-like lesion that is sometimes umbrella shaped after larger volume injections (Figure 1). This likely results from blood rapidly traveling along paths of lower resistance, especially white matter tracts. In contrast to this rapid and stable hematoma, collagenase causes bleeding over hours10 from many burst vessels. Thus, blood appears to infiltrate farther into the parenchyma after collagenase infu-
tion, whereas the tissue is split apart in the blood injection model.

Animal studies should occasionally vary lesion size so that treatment efficacy can be evaluated against a range of insult severities akin to what happens in patients. This is easily done in the collagenase model by varying dose. Different volumes of blood can also be injected, but higher doses affect lesion shape or come back up the needle injection path. Some investigators partly circumvent this through a double injection, where the majority of blood is injected after a small bolus that is allowed to clot.11

Progression and Extent of Injury

Both models have been used to study mechanisms and progression of injury,3 especially in the perihematoma region. Here, injury continues over days or more because of ongoing processes, including blood-brain barrier disruption, edema, and neurotoxicity. This and more distal secondary damage (eg, substantia nigra after striatal ICH10,12), which occurs well after the initial bleed, gives hope that neuroprotectants might limit injury and functional impairment. Accordingly, it would help to know how well animal models predict the amount and rate of cell death in human ICH.

Any comparison of models on perihematoma and secondary injury must account for hematoma volume. Thus, we recently compared models after matching hematoma size, achieved by selecting a dose of collagenase that produced a bleed like that given in the blood injection model.10 Despite this matching, the final lesion size was more than double in the collagenase model, with significantly more perihematoma cell loss, corpus callosum damage, cortical thinning, and distant injury (Figure 1A). Furthermore, repeated imaging from 1 to 6 weeks revealed continuing tissue loss in the collagenase model that did not occur in the whole blood model (Figure 1B). This culminated in considerably greater functional impairment after collagenase infusion. Lesion size findings have been confirmed with histology13 (unpublished data). Thus, very-delayed cell death after collagenase infusion means that there is a wider therapeutic window than in the blood injection model.

Neuroplasticity

There is a plethora of work examining recovery mechanisms after ischemia14 but little on ICH and model comparisons. Although differences in the type, extent, and timing of pathological processes likely mean that recovery processes vary between ischemia and hemorrhage and among models, it makes sense that similar processes occur. For instance, neurogenesis is enhanced after ischemia,14 as well as after ICH in humans15 and the collagenase model.16 Similarly, dendritic plasticity, a key factor in recovery, is affected by ICH. Specifically, after striatal collagenase infusion we found perihematoma dendritic atrophy with eventual normalization, whereas the contralateral striatum showed a sustained increase in branching indicative of synaptogenesis.13 Interestingly, contralateral dendritic branching significantly declines over time in the contralateral striatum after blood injection (unpublished data; Figure 2).

Neuroprotection

There are many putative neuroprotectants for ICH but few comparisons between models. Given the significant benefits of prolonged therapeutic hypothermia (TH; eg, 2 days at 33°C) in global and focal ischemia,17 we tested whether TH improves outcome in rat ICH models (Table 1). We expected considerable benefit because of overlapping mechanisms of injury with ischemia. We also anticipated protection in both ICH models. However, the aforementioned dissimilarities between models, coupled with other mechanistic differences, such as blood-brain barrier disruption10 and inflammation,18 suggest that neuroprotective treatments, including TH, may not work equally well in these models.

Direct comparisons of treatment efficacy in multiple models are potentially confounded by differences in hematoma volume, along with use of somewhat different treatment protocols. Nonetheless, a comparison across studies of TH protocols in the whole blood and collagenase models yields some interesting observations. First, efficacy is considerably greater after ischemia than ICH,17 where proven anti-ischemic protocols only modestly reduce injury and behavioral dysfunction. Second, the benefits of TH vary by ICH model. Early cooling (1-hour delay) aggravates bleeding after collagenase infusion (eg, by coagulopathy), thereby mitigating protection, whereas late cooling (12 hour) is neuroprotec-
This pattern is the opposite of that for ischemia, where earlier is always better, and was not observed in the blood injection model, where TH did not affect bleeding.\textsuperscript{20,21} Regardless, functional improvements with TH are modest despite findings in both models of reduced inflammation, edema, and blood-brain barrier disruption.

In summary, a comparison of TH in these ICH models highlights key differences that hamper predictions of clinical efficacy. The collagenase findings raise concerns with the early use of TH but suggest that neuroprotection is possible when TH is properly timed. Possibly this is from mitigating ongoing cell death and atrophy that occurs in this model and perhaps in humans. Work in the blood injection model suggests that histological benefit will not occur. Finally, these studies suggest that reductions in inflammation and edema may not necessarily translate to improved recovery. This may be a limitation of rodent models, where ICH-induced edema is rarely lethal, and it may question the importance of small reduction in edema. Finally, one should not assume that all neuroprotectants would have model-dependent effects.

**Rehabilitation**

Clinically, many ICH survivors markedly improve despite severe initial disability.\textsuperscript{22} Nonetheless, frequent residual impairments\textsuperscript{1,2} necessitate rehabilitation. Although there are rehabilitation studies in rodents, almost all are in the collagenase model, probably because of its clear long-term behavioral deficits. Simple treatments, such as forced running, improve outcome when started early after striatal collagenase infusion.\textsuperscript{23} However, skilled reaching training appears more effective because it improves recovery and reduces lesion volume even when delayed 1 week post-ICH.\textsuperscript{24,25} We recently applied the same protocol found effective in the collagenase model\textsuperscript{26} to the blood injection model (unpublished data). Although behavioral recovery was enhanced in both models, rehabilitation differentially affected dendritic morphology and lesion volume (Table 2). First, rehabilitation enhanced dendritic growth in contralateral striatum after collagenase but not blood infusion. Second, rehabilitation sometimes reduces tissue loss after collagenase but not blood infusion, an effect likely explained by the much delayed tissue loss that occurs after collagenase infusion. Thus, recovery mechanisms vary with ICH model.

**Summary**

Animal models have greatly improved our understanding of the deleterious and beneficial changes occurring after ICH. Herein, we show some important differences between the 2

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Table 1. Response to Hypothermia in the Whole Blood and Collagenase Models of ICH

<table>
<thead>
<tr>
<th>Variable</th>
<th>Whole Blood</th>
<th>Collagenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding</td>
<td>Unaffected</td>
<td>Exacerbated with early treatment</td>
</tr>
<tr>
<td>Tissue loss</td>
<td>Unaffected</td>
<td>Reduced with delayed treatment</td>
</tr>
<tr>
<td>Edema</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>BBB disruption</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Functional recovery</td>
<td>Modest or no improvement</td>
<td>Modest improvement</td>
</tr>
</tbody>
</table>

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Figure 2. Drawings of representative Golgi-Cox stained neurons in the contralateral striatum after ICH induced by infusion of blood (bottom; unpublished data) or collagenase (top; from Reference 13). Significant dendritic atrophy occurred in the whole blood model but not the collagenase model.
Table 2. Response of the Whole Blood and Collagenase Models to Rehabilitation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Whole Blood</th>
<th>Collagenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue loss</td>
<td>Unaffected</td>
<td>Often reduced</td>
</tr>
<tr>
<td>Contralateral dendritic morphology</td>
<td>Unaffected</td>
<td>Increased length</td>
</tr>
<tr>
<td>Glial response</td>
<td>Not Assessed</td>
<td>Unaffected</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>Not Assessed</td>
<td>Unaffected</td>
</tr>
<tr>
<td>Functional recovery</td>
<td>Improved</td>
<td>Improved</td>
</tr>
</tbody>
</table>

most common rodent models with regard to mechanisms of injury and repair and response to therapeutic interventions. Without an effective clinical intervention, from which one might determine the better model, we recommend that both models be used in the quest for effective ICH therapies.

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Disclosures

None.

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