The Deleterious or Beneficial Effects of Different Agents in Intracerebral Hemorrhage

Think Big, Think Small, or Is Hematoma Size Important?

Richard F. Keep, PhD; Guohua Xi, MD; Ya Hua, MD; Julian T. Hoff, MD

Background and Purpose—Thrombin, heme oxygenase, complement, microglia activation, and leukocyte infiltration are all actively upregulated in intracerebral hemorrhage (ICH). Experimental evidence suggests that all these factors are involved in ICH-induced brain injury. This suggests a scenario whereby ICH actively (through gene and protein upregulation) induces pathways that result in brain injury.

Summary of Review—In this comment, we suggest a potential answer to this conundrum. The upregulation of these factors may have been an evolutionary adaptation to limit brain injury during small hematomas (microbleeds). There is evidence that low levels of thrombin and heme oxygenase limit brain injury. In contrast, the excessive upregulation of these same factors may have a harmful effect after a large hematoma.

Conclusion—The mechanisms upregulated to limit brain injury after microbleeds may also induce injury after large hematomas. The effect of hematoma size on the mechanisms involved in ICH-induced brain injury and the implications of any such effect on clinical therapies merit further investigation. (Stroke. 2005;36:1594-1596.)

Key Words: inflammation • iron • thrombin

During the past decade, animal experimentation has identified a number of pathways involved in intracerebral hemorrhage (ICH)-induced brain injury. Thus, evidence indicates that thrombin, produced during clot formation, is involved in ICH-induced brain edema and neurological deficits. Iron released from hemoglobin via the action of heme oxygenase (HO) is also involved in inducing brain injury, as is an influx of neutrophils, microglia activation, and complement activation. We have also recently found that another iron-containing component of the hematoma, holo-transferrin, in combination with thrombin can induce injury at concentrations, whereas neither alone causes injury.

It is interesting to note that many of the pathways that are implicated in ICH-induced brain injury are actively enhanced during ICH. Thus, whereas thrombin is actively generated during clot formation, there is also a very marked induction of HO-1 after ICH. There is also an influx of neutrophils and other white blood cells into the brain after ICH. The movement of such cells from blood to brain after brain injury involves the upregulation of a variety of molecules involved in the adhesion of white blood cells to the cerebral endothelium, their transmigration, and their direction to the site of injury. Elements of the complement system are upregulated in the perihematomal region, whereas there is extensive microglia activation, with an upregulation of a number of cell surface proteins, especially in aged animals. In the case of the interaction between thrombin and holo-transferrin, it appears that thrombin facilitates the intracellular uptake of iron from holo-transferrin by upregulating levels of the transferrin receptor in parenchymal cells.

In all, these data suggest a scenario whereby ICH actively (in terms of protein upregulation) induces pathways that result in brain injury. This raises the question as to why this should be the case. In this article, we hypothesize that whereas activation of these pathways is deleterious after a large ICH, activation of the same pathways may actually be protective in the setting of small hematomas.

Thus, whereas extensive experimental data has been produced to support these pathways in ICH-induced brain injury, there are also much data indicating that these same pathways can be protective under certain circumstances. Many studies indicate that although high concentrations of thrombin produce deleterious effects on the brain including edema, cytotoxicity, inflammation, and blood–brain barrier breakdown, exposure of the brain to low concentrations can induce protective effects. Pretreatment with low doses of thrombin reduces brain injury in models of ICH, cerebral ischemia, and Parkinson disease. Thrombin can upregulate the level of iron-handling proteins and heat shock proteins that might limit ICH-induced brain injury. Thrombin is also, of course, involved in limiting the size of the ICH through its role in coagulation. Similarly, although there is evidence that

Received January 17, 2005; final revision received April 15, 2005; accepted May 2, 2005.
Correspondence to Richard F. Keep, PhD, Department of Neurosurgery, University of Michigan, 5550 Kresge I, Ann Arbor, MI 48109-0532. E-mail rkeep@umich.edu

Stroke is available at http://www.strokeaha.org

DOI: 10.1161/01.STR.0000170701.41507.e1

1594
HO inhibitors protect in both porcine and rat models of ICH.\textsuperscript{14–16} There is abundant evidence that HO-1 has protective functions.\textsuperscript{17} This enzyme is involved in the breakdown of potentially neurotoxic hemoglobin found in the hematoma and represents an important step in the sequestration of the iron found in hemoglobin.\textsuperscript{2} Evidence suggests that the effects of HO-1 depend on the levels of activity of the enzyme. Thus, Suttner and Denny, using hamster fibroblasts, found that increasing HO-1 expression \textless{}5-fold was protective against oxidative stress, whereas increasing HO-1 expression \textgreater{}15-fold was detrimental.\textsuperscript{17} Similarly, although the influx of neutrophils, activation of microglia, and activation of the complement system appear to be involved in ICH-induced injury, they are also important elements in hematoma resolution. All of these processes contribute to degradation of the hematoma and the damaged perihematoma tissue. This apparent dichotomy between the potential detrimental and beneficial effects of the inflammatory systems has been noted for other forms of brain injury.\textsuperscript{18}

We hypothesize that whether these pathways are protective or deleterious depends on hematoma size. Thus, for example, in a small ICH, thrombin-induced upregulation of the transferrin receptor on parenchymal cells may serve to remove potentially toxic iron from the extracellular space into the intracellular compartment where it can be complexed to molecules such as ferritin (which is also upregulated by thrombin).\textsuperscript{19} However, in a large ICH, thrombin induced iron uptake may exceed the iron-handling capacity of the parenchymal cells and result in brain injury. Similarly, iron-handling proteins in the brain might be sufficient to handle the iron generated by HO in the setting of a small but not a large ICH.

Evolution may have resulted in the development of mechanisms to reduce the limited amount of brain injury that occurs after small hemorrhages. In contrast, there may have been little impact of evolution on generating defense mechanisms against large hemorrhages that would cause death or debilitating injury. The injury from such a large hematoma would still exclude the subject from future procreation or enhancing the survival of previous progeny.

If our hypothesis is correct and the effect of the ICH-induced activation of certain pathways is size-dependent, a major question is at what point(s) do these pathways cease to be protective and start to become deleterious? Another unanswered question is whether the size cutoff between protection and harm will be species dependent? Certainly, the effects of the same volume of ICH will differ between species. A 400-\(\mu\)L hematoma in a rat results in herniation and death but it would have little effect on a human. We suggest, therefore, that it is likely that the hematoma size cutoff between where these pathways are protective and where they are deleterious will be species-dependent, although it is possible that mechanisms are designed to protect only against microbleeds in all species.

Although this essay has focused on intracerebral hemorrhage, it should be noted that there is evidence for an involvement of thrombin, HO, iron, neutrophils, complement, and microglia in ischemic brain damage. Is it possible that certain pathways are activated during small ischemic events to limit brain damage, but that activation of those same pathways during a large ischemic stroke will induce brain damage? In relation to this point, the role of vascular endothelial growth factor (VEGF) in ischemic stroke is intriguing. VEGF is upregulated after ischemic stroke and may limit ischemic brain damage by inducing new blood vessel growth and by direct neuroprotective effects. However, it may also have adverse effects on neuronal survival, blood–brain barrier disruption, and edema formation.\textsuperscript{19} Thus, interestingly, the protective effects of exogenous VEGF are very dose-dependent, with protection at low doses but not at high doses.\textsuperscript{19} It is tempting to postulate that with a small vessel blockage, VEGF may be beneficial by promoting limited new vessel growth and by enhancing neuronal survival. However, a large artery stroke with high levels of VEGF may cause too much angiogenesis with vascular leakage and edema formation.

Though the hypotheses outlined may seem somewhat esoteric, they raise an important issue. If certain ICH-induced pathways result in injury or protection dependent on the size of the hematoma, this would suggest that therapeutic strategies targeting those pathways should only be used for hemmorhages of certain (as yet undefined) size ranges.

Is it possible to test the hypotheses outlined? Much of ICH research has been performed in rodents (rats and, recently, mice). In a rat autologous blood injection model, typically 50 or 100 \(\mu\)L of blood have been infused and thrombin antagonists protect against both hematoma sizes.\textsuperscript{20,21} Thus, at least in terms of thrombin-induced pathways, the hypothesized crossover point between protection and injury would have to occur at hematoma sizes of less (and probably much less) than 50 \(\mu\)L. It is likely that the current methods for assessing ICH-induced injury will have difficulties in assessing damage from such small hematomas (even when protective pathways are inhibited). If we are correct and the hematoma size at which crossover between protection and injury occurs is species-dependent (and possibly dependent on the location of the hematoma), it may be easier to detect protective effects in larger species (such as pig or primate).\textsuperscript{14,22}

In summary, there is evidence that multiple pathways activated during ICH can have both protective and deleterious effects. We hypothesize that these pathways may be protective in the setting of “small” hematomas but have a deleterious effect in the setting of “large” hematomas. The crossover point between protection and injury may be species-dependent. If this hypothesis is correct, it would impact current preclinical experimental design by suggesting that therapeutic agents should be assayed against more than one size of hematoma. It would also suggest that clinical trials might need to focus on a well-defined range of hematoma sizes.

Acknowledgments
This work was supported by grants NS-17760 (J.T.H.), NS-39866, NS-47245 (G.X.), and NS-34709 (R.F.K.) from the National Institutes of Health.

References


The Deleterious or Beneficial Effects of Different Agents in Intracerebral Hemorrhage: Think Big, Think Small, or Is Hematoma Size Important?
Richard F. Keep, Guohua Xi, Ya Hua and Julian T. Hoff

Stroke. 2005;36:1594-1596; originally published online June 2, 2005;
doi: 10.1161/01.STR.0000170701.41507.e1
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/36/7/1594

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/