Hematoma Resolution as a Therapeutic Target

The Role of Microglia/Macrophages

Xiurong Zhao, MD; James Grotta, MD; Nicole Gonzales, MD; Jaroslaw Aronowski, PhD

Abstract—No effective therapy is available for treating intracerebral hemorrhage (ICH). One of several key components of brain damage after ICH is the neurotoxicity of blood products. Within hours to days after ICH, extravasated erythrocytes in the hematoma undergo lysis, releasing cytotoxic hemoglobin, heme, and iron, thereby initiating secondary processes, which negatively influence the viability of cells surrounding the hematoma. To offset this process, phagocytic cells, including the brain’s microglia and hematogenous macrophages, phagocytose and then process extravasated erythrocytes before lysis and subsequent toxicity occurs. Therefore, we hypothesize that a treatment that stimulates phagocytosis will lead to faster removal of blood from the ICH-affected brain, thus limiting/preventing hemolysis from occurring. CD36 is a well-recognized integral microglia/macrophage cell membrane protein known to mediate phagocytosis of damaged, apoptotic, or senescent cells, including erythrocytes. CD36 and catalase expression are regulated by peroxisome proliferator activated receptor-gamma agonists (eg, rosiglitazone). We demonstrate that peroxisome proliferator activated receptor-gamma agonist-induced upregulation of CD36 in macrophages enhances the ability of microglia to phagocytose red blood cells (in vitro assay), helps to improve hematoma resolution, and reduces ICH-induced deficit in a mouse model of ICH. The beneficial role of peroxisome proliferator activated receptor-gamma-induced catalase expression in the context of phagocytosis is also discussed. Peroxisome proliferator activated receptor-gamma agonists could represent a potential treatment strategy for treatment of ICH. (Stroke. 2009;40[Suppl 1]:S92-S94.)

Key Words: catalase ■ CD36 ■ intracerebral hemorrhage ■ phagocytosis ■ PPARγ

Intracerebral hemorrhage (ICH) accounts for 10% to 15% of all strokes and has a 1-year mortality rate greater than 50% to 60%.1,2 There is currently no US Food and Drug Administration-approved treatment for ICH.

Although the majority of damage may occur within the first few hours after ICH due to the mass effect of the hematoma, a secondary cause of injury is due to the presence of intraparenchymal blood. The nature of this secondary damage is complex, but it is caused primarily by the cytotoxic effect of extravasated blood and by cytotoxic substances released by activated neuroglia and hematogenous cells that invade the brain.3,4 This cytotoxic insult has a strong oxidative component and ultimately leads to neuronal loss, gray matter damage, vascular injury, blood–brain barrier disruption, and deadly brain edema.3–10 Because the presence of intraparenchymal blood is the source of cytotoxic insult and inflammation, we propose that secondary damage to the brain after ICH could be reduced by augmenting removal of the intraparenchymal blood and anti-inflammatory or cytoprotective agents.

We propose that peroxisome proliferator–activated receptor-gamma (PPARγ), a transcription factor and pleiotropic mediator for cellular defense (cytoprotection) and a stimulator for the scavenging system (hematoma clearance), may represent a novel target for ICH therapy.11 Hence, the central hypothesis is that PPARγ, through mechanisms which include upregulation of CD36 (the phagocytosis-facilitating gene), promotes hematoma clearance. Faster hematoma resolution prevents secondary damage caused by the toxicity of the hematoma and hematoma-induced inflammation.

In addition, PPARγ acts as a key genomic homeostatic regulator for intracellular stress by promoting the transcription of gene products that have a key role in antioxidative defense such as catalase12 and superoxide dismutase,13 which help not only to improve neuronal resistance, but also protect microglia from damage, thus preserving their phagocytic (hematoma clearance) functions.

Peroxisome Proliferator–Activated Receptor-Gamma Upregulates CD36 and Promotes Microglia-Mediated Phagocytosis in Culture

Phagocytosis mediated by microglia and macrophages at the site of brain injury is coordinated by a highly complex set of proteins that mediates anchoring, internalization, and processing of the phagocytic targets. One well-recognized

Received and accepted July 30, 2008.
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Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.108.533158
The process of phagocytosis is facilitated by the scavenger receptor CD36, an integral protein component of the phagocyte membrane. Phagocytosis is associated with the generation of reactive oxygen species (ROS) and oxidative stress, which is deleterious to all brain cells as well as to the phagocyte itself. Rosiglitazone or prostaglandin 15d-PGJ₂-mediated activation of the transcription factor PPARγ induces expression of CD36 and catalase in phagocytes. Increased expression of CD36 improves phagocytic capacity, but also increases oxidative stress due to the augmented phagocytosis. Cytoprotection remains possible due to the increased expression of catalase, which ameliorates oxidative stress and reduces damage to the brain cells, including preservation of phagocyte integrity and function to allow for more effective hematoma resolution (phagocytosis).

Figure. RBCs represent the main component of the hematoma and are removed from the brain parenchyma by phagocytes such as microglia and hematogenous macrophages. The process of phagocytosis is facilitated by the scavenger receptor CD36, an integral protein component of the phagocyte membrane. Phagocytosis is associated with the generation of reactive oxygen species (ROS) and oxidative stress, which is deleterious to all brain cells as well as to the phagocyte itself. Rosiglitazone or prostaglandin 15d-PGJ₂-mediated activation of the transcription factor PPARγ induces expression of CD36 and catalase in phagocytes. Increased expression of CD36 improves phagocytic capacity, but also increases oxidative stress due to the augmented phagocytosis. Cytoprotection remains possible due to the increased expression of catalase, which ameliorates oxidative stress and reduces damage to the brain cells, including preservation of phagocyte integrity and function to allow for more effective hematoma resolution (phagocytosis).

Peroxisome Proliferator–Activated Receptor-Gamma Agonist Improves CD36 and Catalase Expression, Hematoma Resolution, and Neurological Deficit After Intracerebral Hemorrhage

We produced ICH by injecting 15 μL of autologous blood into a mouse brain. Mice were treated with rosiglitazone 24 hours after ICH and continued for 14 days (once a day). This treatment significantly increased expression of CD36 and catalase in the perihematoma brain (as determined 3 days after ICH), improved hematoma resolution (as determined by measuring the brain’s hemoglobin and morphometrically and at 7 and 14 days) and improved functional deficit (determined using behavioral tests).

Sources of Funding

This project was supported by R01NS052791, 1R21NS057284, and R01NS060768 grants from National Institutes of Health/National Institute of Neurological Diseases and Stroke.

Disclosures

None.

References

6. Huang FP, Xi G, Keep RF, Hua Y, Nemoianu A, Hoff JT. Brain edema and the phagocytes at an increased risk of oxidative stress. In our laboratory, we observed that microglia in culture are more efficient in conducting phagocytosis of RBCs when exogenous catalase is added to the culture media. Catalase is also upregulated when rosiglitazone or 15d-PGJ₂ is added to the microglia-containing media. This catalase helps to limit oxidative stress as demonstrated by a significant reduction in the amount of hydrogen peroxide in culture media from a dish containing microglia involved in RBC phagocytosis in the presence of rosiglitazone or 15d-PGJ₂ compared with vehicle control. We also demonstrated that such inhibition of pro-oxidative behavior of microglia during phagocytosis by PPARγ agonists prevents microglia from injuring neurons (coculture experiment) and themselves.

Peroxisome Proliferator–Activated Receptor-Gamma Agonists Upregulate Catalase and Reduce the Oxidative Stress That Inhibits Phagocytosis

Phagocytosis followed by degradation and processing of phagocytic targets in phagolysosomes generates a large quantity of reactive oxygen species and places brain cells...


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Stroke. 2009;40:S92-S94; originally published online December 8, 2008;
doi: 10.1161/STRKEAHA.108.533158
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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/40/3_suppl_1/S92