Hematoma Resolution as a Therapeutic Target
The Role of Microglia/Macrophages

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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.5–10 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
component of the phagocytic apparatus is a cell membrane protein CD36, a class B scavenger receptor. The presence of CD36 is important for the process of phagocytosis. Transfection of nonphagocytic cells with the CD36-expressing gene converts the nonphagocytic cell into a phagocyte. CD36 gene expression is controlled by the transcription factor PPARγ. In our laboratory, we tested whether PPARγ agonists such as rosiglitazone and pioglitazone (US Food and Drug Administration-approved antidiabetic drugs) or the endogenous PPARγ agonist 15-deoxy-delta-12,14-prostaglandin J2 could increase CD36 expression in microglia and whether this expression could transform microglia into more efficient phagocytes of red blood cells (RBCs) with an in vitro (culture dish) model for hematoma clearance. In agreement with these assumptions, rosiglitazone and 15d-PGJ2 increased expression of CD36 and promoted phagocytosis of RBCs by microglia, whereas PPARγ inhibition (achieved through pharmacological antagonist GW9662 or adenovirus-mediated PPARγ gene knockdown) reduced CD36 expression and slowed down phagocytosis of RBCs by microglia. The functional relevance of CD36 was demonstrated by showing that anti-CD36 antibody inhibited PPARγ agonist-mediated phagocytosis.

**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 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 experiments) and themselves.

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**Disclosures**

None.

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