Molecular Pathophysicsiology of Cerebral Hemorrhage
Secondary Brain Injury

Jaroslaw Aronowski, PhD; Xiurong Zhao, MD

Abstract—Intracerebral hemorrhage (ICH) is an often fatal type of stroke that kills ≈30,000 people annually in the United States. If the patient survives the ictus, then the resulting hematoma within brain parenchyma triggers a series of adverse events causing secondary insults and severe neurological deficits. This article discusses selected aspects of secondary brain injury after ICH and outlines key mechanisms associated with hematoma toxicity, oxidative stress, and inflammation. Finally, this review discusses the relevance of hematoma resolution processes as a target for ICH therapy and presents potential clinically relevant molecular targets that could be harnessed to treat secondary injury associated with ICH injury. (Stroke. 2011;42:1781-1786.)

Key Words: hematoma clearance ■ hematoma toxicity ■ intracerebral hemorrhage ■ oxidative stress

 dùng intracerebral hemorrhage (ICH), rapid accumulation of blood within brain parenchyma leads to disruption of normal anatomy and increased local pressure. Depending on the dynamic of hematoma expansion (growth), the primary damage occurs within minutes to hours from the onset of bleeding and is primarily the result of mechanical damage associated with the mass effect. Secondary damage is, for the most part, attributable to the presence of intraparenchymal blood and may be dependent on the initial hematoma volume, age, or ventricular volume. It may occur through many parallel pathological pathways, including: (1) cytotoxicity of blood;2,3 (2) hypermetabolism;4 (3) excitotoxicity;5 (4) spreading depression;6 and (5) oxidative stress and inflammation.3,7–13 Ultimately, this pathogenesis leads to irreversible disruption of the components of the neurovascular unit, constituting gray and white matter, and is followed by blood—brain barrier disruption and deadly brain edema with massive brain cell death.2,3,5,7,14–16 Whereas inflammatory mediators generated locally in response to brain death/injury have the capacity to augment damage caused by ICH (secondary injury), the involvement of inflammatory cells, eg, microglia/macrophages, is vital for removal/cleanup of cellular debris from the hematoma, the source of ongoing inflammation.17 The timely removal of damaged tissue is essential for reducing the length of deleterious pathological process and thereby allowing for faster and more efficient recovery.

Blood Cytotoxicity and Oxidative Stress as Mediators of Cell Death After ICH

After ICH, the extravasated blood components (primarily erythrocytes and plasma proteins) and the damage-associated molecular patterns, including nucleic acids, extracellular matrix components, proteins, lipid mediators, ATP, and uric acid released from necrotic and damaged tissue, impose a strong cytotoxic, pro-oxidative, and proinflammatory insult toward adjacent viable brain cells and could be seen as early as minutes after onset of ICH. At this early stage, the toxicity of extravasated blood plasma components including blood-derived coagulation factors, complement components, immunoglobulins, and other bioactive molecules is proposed to act as contributors to ICH-affected tissue damage.2–3,11 Subsequently, red blood cell lysis starts at ≈24 hours and continues for the next several days, leading to release of cytotoxic hemoglobin (Hb) with further deterioration of the pathological status quo.2 Hb and its degradation products, heme and iron, directly compromise the well-being of neighboring brain cells.2,18–20 Hb and heme are potent cytotoxic chemicals capable of causing death to many brain cells. Prominently, the mechanism of Hb toxicity is via generating free radicals (mainly through Fenton-type mechanism) and massive oxidative damage to proteins, nucleic acids, carbohydrates, and lipids.2,7,18,21,22 Therefore, finding means of controlling hemolysis, and detoxification, and absorption of Hb, heme, and iron (Figure 1) may have important clinical implications. We review the role of selected pathways involved in blood detoxification process, including transcriptional regulation of selected molecules with a role in hematoma clearance.

Haptoglobin and Hemopexin Acts to Combat Hb/Heme Toxicity After ICH

Haptoglobin (Hp), an acute phase protein, is an abundant blood plasma component that is normally synthesized and released into blood circulation primarily by hepatocytes but...
to a lesser extent by lungs, kidneys, skin, and adipose tissue. Hp is a heteromorphic enzyme composed of alpha and beta chains. The primary function of Hp in blood is to bind and neutralize nephrotoxic free Hb in case of intravascular hemolysis. Hp–Hb complexes are consequently removed from the circulation by a specialized subclass of macrophages expressing CD163, a scavenger receptor and a member of the group B scavenger receptor cysteine-rich superfamily. Under normal circumstances, Hp represents an effective mechanism by which our body is protected from Hb toxicity. However, because Hp synthesis is not increased by low Hp levels and Hp is not recycled by macrophages, it may take 5 to 7 days for the Hp level to recover if completely sequestered by Hb. Thus, massive hemolysis may lead to persistent hypohaptoglobinemia. Interestingly, we have recently demonstrated that Hp is also produced locally in rat brain after ICH and its expression is significantly increased around the hematoma within hours from the onset of ICH.

The brain-derived Hp appeared to be synthesized and released by oligodendrocytes. Because oligodendroglia are abundant in white matter and are present throughout the gray matter, local production of Hp by these cells likely represents an important endogenous mechanism protecting brain against the extravascular Hb toxicity. Indirect support for such a claim includes (1) primary oligodendrocytes protect neurons in culture from Hb toxicity via Hp release; (2) animals made hypohaptoglobinemic with repetitive Hb administration, before ICH, experience more extensive brain damage; (3) mice genetically engineered to overexpress Hp are less susceptible to ICH injury; and (4) Hp-deficient mice are more vulnerable to ICH injury. In the context of therapeutic relevance, we have determined that pharmacological intervention with sulforaphane, a naturally occurring agent that acts as NF-E2–related factor-2 (Nrf2) transcription factor activator, increases Hp in blood plasma and brain, and notably reduces brain damage in animal models of ICH.

In humans, the Hp gene exists in 2 major allelic forms designated as Hp1 and Hp2. Hp2 is produced via intragenic duplication of a 1.7-kb DNA fragment of Hp1 gene. Consequently, 3 major Hp genotypes are formed, Hp1-1, Hp1-2, and Hp2-2. The largest of the 3 Hp genotypes, has the lowest Hb-binding activity (Hp1-1 > Hp1-2 > Hp2-2) and, because of its size, represents potentially less bioavailable moieties. This may lead to a more defective Hb clearing system. Interestingly, according to epidemiological studies, patients carrying the Hp2-2 genotype demonstrate more severe vasospasm after subarachnoid hemorrhage, more positive association with idiopathic seizures (and possibly post-traumatic epilepsy), and higher incidence of carotid atherosclerosis in diabetic patients. The relevance of Hp genotype as a factor modifying the outcome after ICH has not been studied to date.
It could also be relevant for this review to indicate that in addition to the Hp-Hb/CD163 scavenging system, an independent system exists to help remove Hb breakdown products, heme, and iron. Hemopexin (Hx) is a blood plasma glycoprotein synthesized primarily by hepatocytes. Hx has been shown to bind to heme with a high affinity and forms stable Hx-heme complexes. The heme–Hx complexes are readily endocytosed by macrophages expressing CD91 (α2-macroglobulin receptor, also known as low-density lipoprotein receptor-related protein-1). Although under physiological conditions CD91 plays a role in recycling iron in response to extravascular hemolysis in hematoma-affected tissue, the Hx-heme/CD91 system may facilitate removal of pro-oxidative heme by microglia/macrophages. Recent studies and ongoing research in this laboratory support this notion and suggest that Hx-deficient mice experience augmented ICH injury. More studies of the role of Hx in ICH are warranted.

Heme Oxygenase in ICH
Phagocytosis of intact erythrocytes, as well endocytosis of Hb and heme by macrophages/microglia, are instrumental for removing the pro-oxidative heme from the extracellular space of brain parenchyma. Phagocytic cells involved in this process express heme oxygenase (HO), a rate-limiting enzyme involved in heme catabolism that converts heme to biliverdin, carbon monoxide, and iron (FeII). There are 2 isoenzymes of HO; HO1 (also called HSP32) is an inducible enzyme involved in heme catabolism and HO2 is constitutively expressed. Whereas HO2 is moderately abundant in most cell types, including neurons, HO1 expression after ICH is primarily induced in endothelial cells and microglia/macrophages. Based on studies using genetically engineered mice, it was determined that HO2-null mice are more susceptible, whereas HO1-null mice are more resistant to ICH-mediated damage, as compared to wild-type mice. The improved outcome in the HO1-null mice after ICH is a surprise, because HO1 deficiency in many other brain injury models including ischemic stroke are associated with augmented brain damage. One potential explanation for such discrepancy is that HO1 deficiency could reduce the excessive liberation of free iron from erythrocytes in hematoma (feature that is unique to ICH) and consequently limit the iron-mediated oxidative stress. Although this scenario may provide logistics for reduced ICH injury in HO1-deficient mice, the presence of increased levels of Hp and Hx in HO1-deficiency animals needs to be acknowledged, because these factors may contribute to cytoprotection independently of HO-1. Additional limitation regarding interpretation of the data on HO1-null mice susceptibility to ICH injury is that the outcome was characterized only up to 3 days and it did not address the role HO1 could play at later stages when a majority of hemolysis and clean-up takes place. This timing issue is of particular importance because HO1 deficiency makes phagocytic cells, the main executors of brain cleanup process, more prone to self-injury on processing of engulfed heme-containing red blood cell. After ICH in mice, the clean-up process may last 3 to 4 weeks.

Another intriguing finding is that a robust HO1 upregulation in ICH-affected brain in response to treatment with sulforaphane coincides with a significant reduction of brain damage. Sulforaphane promotes HO1 expression via Nrf2 through antioxidant response element. Interestingly, porphyric derivatives that can be used to inhibit HO1, in contrast to sulforaphane, showed reduced ICH damage. Thus, more studies are required to clarify the role of HO1 in ICH.

Oxidative Stress and ICH Injury
As indicated, oxidative stress appears to play a prominent role in ICH pathogenesis. Direct evidence for the causal relationship between free radicals and ICH injury was by demonstrating the efficacy of antioxidants as therapeutic agents. Specifically, the free radical scavengers, such as dimethylthiourea, α-phenyl-N-tert-butyl nitrone, NXY-059 (a sulfonil derivativ of α-phenyl-N-tert-butyl nitrone) or deferoxamine, a drug chelating pro-oxidative iron, significantly reduced brain injury in animal models of ICH. In agreement with these pharmacological experiments, mice with genetically deleted NADPH oxidase, a key enzyme involved in generating reactive oxygen species, showed reduced damage after ICH. It recently has been demonstrated that estrogen reduces ferrous iron toxicity in vivo and in vitro, indicating that gender difference in susceptibility to ICH may, in part, be associated with differences in handling iron toxicity.

Although preclinical evidence exists for efficacy of therapy based on free radical neutralization, clinical trial with NXY-059 was surprisingly disappointing. NXY-059 was not only ineffective in ischemic stroke but also not beneficial in ICH patients. The reason for these negative outcomes is unclear; however, factors such as pharmacokinetics of the drug (no blood–brain barrier permeability) and inability to stoichiometrically neutralize high levels of free radicals could likely contribute to these neutral results.

Deferoxamine and Iron Detoxification
Deferoxamine is another antioxidant currently in the early promising stages of clinical trial. As indicated, toxicity of free iron originated from extravascular hemolysis, and HO-mediated catabolism is well-documented. Iron (II), by reacting with H2O2 generates hydroxyl radicals and deferoxamine, by forming stable complexes with iron prevents its engagement in oxidative reactions. One caveat exists that deferoxamine may act not only through preventing iron-mediated pro-oxidative catalyzes but also via inhibiting prollyl hydroxylase activity (enzyme using iron as cofactor), which leads to HIF1α availability, a pathway with neuroprotective effect. Regardless of its mechanism, preclinical studies in a rat and pig ICH model showed promise for deferoxamine as therapy. Recently, a phase I study assessing the feasibility, safety, and maximum tolerated dose of deferoxamine in ICH patients was successfully completed (Magdy Selin, personal communication).

Role of Nrf2 in Oxidative Damage
In addition to increased free radical generation, damage to brain tissue may result from the impairment of the endogenous antioxidative enzyme system in response to ICH. Hua et al reported a significant reduction in the levels of manga-
nese superoxide dismutase and copper/zinc superoxide dismutase, the key enzymes of the antioxidant defense system in brains, after intracerebral injection of lysed erythrocytes. Thus, because the antioxidant capacity of ICH-affected brain may be deficient, strategies based on stimulating endogenous antioxidant capacity of the brain could offer an effective therapeutic approach. To test this hypothesis, we and others took advantage of ubiquitous transcription factor, Nrf2, a key player in antioxidative homeostasis. By binding to the antioxidant response element, Nrf2 regulates the expression of many detoxification and antioxidant enzymes, including superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase, HO-1, NAD(P)H quinone oxidoreductase-1, peroxiredoxin, or thioredoxin. To activate Nrf2 in animals after ICH, we again utilized a naturally occurring organosulfur compound, sulforaphane. As expected, treatment with sulforaphane effectively increased the expression of Nrf2-regulated antioxidant genes, including catalase, superoxide dismutase, and glutathione-S-transferase, in brain tissue after ICH. Notably, this expression of antioxidants corresponded to reduced oxidative damage to proteins and lipids within the ICH-affected brain and, importantly, with less severe neurological deficits. To produce ultimate evidence for the beneficial involvement of Nrf2 in ICH pathogenesis, we established that mice deficient in Nrf2 (knockout) displayed more severe neurological deficit when subjected to ICH, implying that Nrf2 plays critical safeguard function in defending brain against oxidative stress associated with ICH pathogenesis.

Cytoprotective Effect of Peroxisome Proliferator-Activated Receptor-γ

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-dependent transcription factor that regulates target gene expression by binding to conserved DNA sequences termed peroxisome-proliferator response elements as heterodimers with another nuclear receptor, the retinoic acid receptor. In addition to a well-characterized regulation of expression of genes involved in lipid and glucose metabolism and energy storage, activation of PPARγ induces expression of antioxidative catalase and copper/zinc superoxide dismutase, two ubiquitous enzymes capable of alleviating oxidative stress by decomposing hyperactive H2O2 into molecular oxygen and water molecules, and dismutation of superoxide into oxygen and H2O2, respectively. The ligands for PPARγ include fatty acids, nonsteroidal anti-inflammatory drugs, 15Deoxy-D12,14-Prostaglandin J2 (15D-PGJ2, a reactive membrane lipid metabolite and downstream product of prostaglandin D2, proposed to act as a physiological PPARγ agonist), and the thiazolidinediones (specifically pioglitazone and rosiglitazone), a class of compounds commonly used to treat type 2 diabetes. Using rat model of ICH based on intrastriatal autologous blood injection, we recently reported that intrahemorrhage injection of 15D-PGJ2 activates PPARγ in the brain tissue and increases expression of catalase in ICH-affected brain, primarily in neurons and microglia. These biochemical responses were associated with decreased neuronal death, as demonstrated by the incidence of DNA scission in neurons, and reduced neurological dysfunction. These findings are consistent with studies demonstrating that PPARγ plays an important role in protecting neurons and other brain cells from injury caused by oxidative stress and ischemia.

Inflammation, Blood Toxicity, and Hematoma Removal as Target for ICH Treatment

The brain-resident phagocytes, microglia, are highly abundant (10%–15% of total glial cells) in brain and become readily activated within minutes after ICH. The activated microglia release proinflammatory cytokines and chemotactic factors, which help to recruit hemotogeneous inflammatory cells to the ICH injury sites. This is characterized first by the transient (18 hours–4 days) infiltration of neutrophils and then a long-term (1 day–months) presence of hematogenous macrophages. The inflammatory signaling involves a coordinated effort of different molecules and cell types and is largely coordinated by a ubiquitous transcription factor, nuclear factor kappa-B (NF-κB). The gene targets of NF-κB include various adhesion molecules (including these involved in immune cells extravasation, eg, intercellular adhesion molecule-1), cytokines, and chemokines (involved in proinflammatory signaling and NF-κB activation, eg, IL-1β and tumor necrosis factor-α), metalloproteinases (eg, matrix metalloproteinase-9), immune receptors, acute phase proteins, cell surface receptors, and inflammatory enzymes (eg, inducible nitric oxide synthase, COX-2, and PLA2). It is important to mention that free radicals act as important signaling molecules in NF-κB activation. This property of NF-κB may, in part, explain how oxidative stress could enhance inflammation after ICH. Experimental studies demonstrate that NF-κB is activated in ICH-affected hemisphere as early as 15 minutes after the onset of ICH, reaches maximum between 1 and 3 days, and remains elevated for weeks. It is pertinent to indicate that NF-κB target genes including IL-1β, tumor necrosis factor-α, and matrix metalloproteinase-9 are involved in ICH-mediated brain injury.

Albeit some inflammatory responses generated by microglia/macrophages after ICH may aggravate brain injury, microglia/macrophages-mediated phagocytosis is instrumental in conducting brain clean-up, the process that must occur to allow for tissue repair and functional recovery. A fast and efficient removal of apoptotic, dislocated (eg, extravascular erythrocytes), and damaged cells before the discharge of injurious and proinflammatory cell contents (damage-associated molecular patterns) occurs and may help to reduce secondary damage. Microglia and macrophages express various cell surface receptors, including scavenger receptors (eg, CD91, scavenger receptor-A, MARCO, scavenger receptor-BI, and CD36) that assist in phagocytosis/endocytosis-mediated removal of cellular debris after tissue injury, including brain injury after ICH. One specific study evaluated CD36, a class II scavenger receptor that is transcriptionally regulated by PPARγ. This study used in vitro and in vivo models and demonstrated that: (1) microglia/macrophages utilize CD36 to promote phagocytosis of red blood cell; and (2) treating animals with PPARγ agonists (eg, rosiglitazone, pioglitazone, or 15D-PGJ2), which increased CD36 expression, results in faster hematoma resolu-


Molecular Pathophysiology of Cerebral Hemorrhage: Secondary Brain Injury
Jaroslaw Aronowski and Xiurong Zhao

*Stroke*. 2011;42:1781-1786; originally published online April 28, 2011; doi: 10.1161/STROKEAHA.110.596718

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 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/42/6/1781

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2012/03/12/STROKEAHA.110.596718.DC1

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/
Molecular Pathophysiology of Cerebral Hemorrhage Secondary Brain Injury

Jaroslaw Aronowski, PhD; Xiurong Zhao, MD

(Stroke. 2011;42:1781-1786.)

Key Words: hematoma clearance ■ hematoma toxicity ■ intracerebral hemorrhage ■ oxidative stress
ICH is a type of hemorrhage that can be caused by various factors, including trauma, hypertension, or vascular anomalies. The release of hemoglobin (Hb) into the brain parenchyma leads to an oxidative stress response, which can cause further damage to brain cells. The figure illustrates the role of macrophages in the clearance of hemoglobin and the importance of haptoglobin (Hp) in the binding of hemoglobin to prevent its toxic effects.

**Figure 1.** During intracerebral hemorrhage (ICH), blood is released into the brain matter. Erythrocytes (red blood cells) are cleared from the parenchyma by microglia/macrophages through cell-surface scavenger receptor CD36-mediated phagocytosis. Timely clearance of the extravasated red blood cells and irreversibly injured cells prevents them from undergoing lyses and subsequent spillage of toxic contents into the brain parenchyma. In the case of hemolysis (what unavoidably occurs after ICH), hemoglobin (Hb) needs to be removed quickly from the extracellular space to avoid its cytotoxic effects. Haptoglobin (Hp), a protein arriving to the brain from blood and synthesized locally by oligodendroglia, tightly binds Hb, forming less toxic Hb–Hp complexes that are endocytosed by microglia/macrophages through scavenger receptor CD163. The toxic extracellular free heme generated from Hb can be neutralized by binding to hemopexin (Hp). The heme–Hx complexes are subsequently removed by phagocytes via CD91 scavenger receptor-mediated endocytosis. In phagocyte, heme is metabolized by heme oxygenase (HO; primarily, HO-1) to biliverdin, carbon monoxide (CO), and pro-oxidative iron. To prevent oxidative cell damage, iron is sequestered within phagocyte by iron-binding proteins such as hemosiderin or ferritin. Excessive production of iron may saturate storing capacity of hemosiderin, leading to oxidative injury from the free iron. CD36 is expressed under control of peroxisome proliferator-activated receptor-gamma (PPARγ) and Hx expression is increased with NF-E2–related factor-2 (Nrf2). Both Nrf2 and PPARγ increase expression of antioxidant proteins (eg, catalase or superoxide dismutase). Thus, activation of these transcription factors may represent new targets for ICH treatment.

**ICH** refers to intracerebral hemorrhage, which can be caused by various factors, including trauma, hypertension, or vascular anomalies. The release of hemoglobin (Hb) into the brain parenchyma leads to an oxidative stress response, which can cause further damage to brain cells. The figure illustrates the role of macrophages in the clearance of hemoglobin and the importance of haptoglobin (Hp) in the binding of hemoglobin to prevent its toxic effects.

**Figure 1.** During intracerebral hemorrhage (ICH), blood is released into the brain matter. Erythrocytes (red blood cells) are cleared from the parenchyma by microglia/macrophages through cell-surface scavenger receptor CD36-mediated phagocytosis. Timely clearance of the extravasated red blood cells and irreversibly injured cells prevents them from undergoing lyses and subsequent spillage of toxic contents into the brain parenchyma. In the case of hemolysis (what unavoidably occurs after ICH), hemoglobin (Hb) needs to be removed quickly from the extracellular space to avoid its cytotoxic effects. Haptoglobin (Hp), a protein arriving to the brain from blood and synthesized locally by oligodendroglia, tightly binds Hb, forming less toxic Hb–Hp complexes that are endocytosed by microglia/macrophages through scavenger receptor CD163. The toxic extracellular free heme generated from Hb can be neutralized by binding to hemopexin (Hp). The heme–Hx complexes are subsequently removed by phagocytes via CD91 scavenger receptor-mediated endocytosis. In phagocyte, heme is metabolized by heme oxygenase (HO; primarily, HO-1) to biliverdin, carbon monoxide (CO), and pro-oxidative iron. To prevent oxidative cell damage, iron is sequestered within phagocyte by iron-binding proteins such as hemosiderin or ferritin. Excessive production of iron may saturate storing capacity of hemosiderin, leading to oxidative injury from the free iron. CD36 is expressed under control of peroxisome proliferator-activated receptor-gamma (PPARγ) and Hx expression is increased with NF-E2–related factor-2 (Nrf2). Both Nrf2 and PPARγ increase expression of antioxidant proteins (eg, catalase or superoxide dismutase). Thus, activation of these transcription factors may represent new targets for ICH treatment.

**ICH** refers to intracerebral hemorrhage, which can be caused by various factors, including trauma, hypertension, or vascular anomalies. The release of hemoglobin (Hb) into the brain parenchyma leads to an oxidative stress response, which can cause further damage to brain cells. The figure illustrates the role of macrophages in the clearance of hemoglobin and the importance of haptoglobin (Hp) in the binding of hemoglobin to prevent its toxic effects.

**ICH** refers to intracerebral hemorrhage, which can be caused by various factors, including trauma, hypertension, or vascular anomalies. The release of hemoglobin (Hb) into the brain parenchyma leads to an oxidative stress response, which can cause further damage to brain cells. The figure illustrates the role of macrophages in the clearance of hemoglobin and the importance of haptoglobin (Hp) in the binding of hemoglobin to prevent its toxic effects.
서 생성된 Hp는 회소도기아교세포(oligodendrocyte)에서 혈성되어 유리되는 것으로 보인다. 회소도기아교세포는 백색질에 속하며 회색사이에도 분포하기 때문에, Hp의 소교성생산은 혈관 외로 유출된 Hp의 독성에 대항하는 중요한 내세척으로 거부가 되어 생성된다. 이러한 주장은 퇴치학적 간접적인 증거로, (1) 뇌척수액 내 수지환원을 통한 HP가 독성으로부터 신경세포를 보호하고, (2) 변분적 HP가 입을 통해 젊은혈액판정증의 유도한 게시는 ICH 이후보다 뉴소성이 희박해지며, (3) 유전적 조작을 통하여 Hp를 과발현하는 마우스는 ICH에 의한 손상이 상당적으로 적고, (4) Hp-결론 마우스는 ICH에 의한 조직 손상에 보다 민감하다는 것이다.43 치료적 관점에서 저자들은 NF-E2 관련 요소-2 (Nrf2) 전사 요소 활성체이며 자연적으로 존재하는 물질인 sulforaphane줄을 둘러싼 경우 혈액 내 콜라주스(blood plasma) 및 뇌에서 HP가 증가하며, 18 ICH의 동물 모델에서 뉴소성이 감소함을 밝혔다.44


본 증상을 통하하여 Hp/Hb/CD163 척소 시스템 이외에도 Hp 분해 산물, heme 및 혈분을 제거하는 별도의 시스템이 있음을 지적하는 것도 적절한 것이다. 26 혈모세린(hemopexin, Hx)은 주로 간세포에서 형성되는 혈액 내 플라즈마 단단체질이다. 26 Heme-Hx 복합체는 CD91 (α2-macroglobulin 수용체, 또한 저밀도질단백질 수용체 관련 단백질-1 [low-density lipoprotein receptor-related protein-1])으로도 알려져 있음을 발견하는 대식세포에 의하여 바로 세포 내 섭취(endocytosis)된다. 생리적 상태에서 CD91은 혈중이 발생한 조직에서 혈관 외 용해에 의한 침범을 제한하는 기능을 가진다. Hx-heme/CD91 시스템은 미세아교세포/대식세포 등에 의한 산화성 heme의 제거를 촉진하는 기능을 한다. 25 본 연구팀이 수행한 최근의 연구26 및 현재 진행 중인 연구 결과를 통해 이 가설이 증명되고 있으며, Hx가 결핍되는 마우스는 ICH에 의한 손상이 증가할 것으로 보인다. ICH에서 Hx의 역할에 대하여 더 많은 연구가 필요하다.

ICH에서의 heme 산화효소

정상적인 적혈구의 다식(phagocytosis) 및 대식세포/미세아교세포에 의한 Hb와 heme의 세포 내 섭취는 심장의 세포 내 공간에서 산화성 손상을 유발하는 heme를 제거하는 기전으로 사용된다. 이러한 과정에 관여하는 대식세포는 heme 산화 효소(heme oxygenase, HO)를 발현하며, 이는 heme를 biliverdin, 일산화탄소 및 철(FeII)로 변화시키는 heme 대사

의 속도 제한 단계(rate-limiting step)이다.20,21 HO의 동종효소는 2가지가 있다, HO1 (HSP32로도 불린다)는 유도형이며, HO2는 항상 발현되는 형태이다.20,21 HO1는 신경세포에 포함된 모든 종류의 세포에서 비교적 풍부하게 발견되는 반면, ICH 이후 HO1 발현은 주로 내피세포 및 미세아교세포/대식세포에서 유도된다. 유전자 조작 마우스를 사용한 연구에서, HO2를 발현하지 않는 마우스는 ICH 이후 조직 손상에 더욱 취약하였다. 20 그러나 HO1를 발현하지 않는 마우스는 정상 마우스(wild-type)에 비하여 ICH 이후 조직 손상을 잘 받지 않았다. 21 ICH 이후 조직 손상에 HO1을 발현하지 않는 마우스가 더욱 취약하다는 것은 매우 놀라운 발견인데, 이는 HO1 결핍이 허혈성뇌증을 포함하여 다른 여러 뉴소성 모델에서 조직손상 증가와 관련되어 있었기 때문이다. 그러한 차이에 대한 한 가지 설명으로, HO1 결핍은 혈중 내의 적혈구로부터 절만이 유도되는 것을 감소시키는 것이며(이러한 현상은 ICH에 고유한 것이다) 따라서 결과적으로 첫번에 의한 산화성 손상을 막을 수 있다는 가설이 제거되었다. 이 시나리오가 HO1-결핍 마우스에서 ICH에 의한 뉴소성 손상 감소를 설명하는 하나, HO1-결핍 동물에서 Hp 및 Hx의 농도가 증가하여 이들에 의한 세포보호 효과가 개선되어 있을 가능성 또한 고려해야 한다. 또한 HO1-결핍 마우스에서의 ICH 실험을 해석할 때 유의해야 할 또 다른 점은, 피로하의 실험이 조직 손상을 ICH 유도 후 3일 시점에서 측정하였다는 것이며, 따라서 용혈 현상 및 이후 혈관 손상의 제거 과정이 활성화될 만성기에서 HO1의 역할에 대해서는 알 수 없다는 사실이다. HO1 결핍은 뉴에서 척소 작용을 주로 담당하는 대식세포가 HO1 결핍에 의하여 대식 heme-항염적혈구에 의한 자가 손상에 더 취약하다는 점을 고려하면, 허혈 이후 조직 손상 정량 시점은 중요한 문제라고 할 수 있었다. 21 마우스에서 ICH가 발생한 이후, 척소 과정은 3~4주 이후까지 지속되며 알려져 있다. 21

또 다른 흙소로운 발견으로 ICH를 유도한 마우스에 sulforaphane를 투여하면 뉴조직에서 HO1이 현저히 증가하는데, 이때 뉴조직 손상 역시 감소한다는 결과이다. 26 Sulforaphane는 Nr2를 통하여 HO1 발현을 촉진하는데, 항산화 반응을 유발하는 과정을 거친다고 알려져 있다. 또한, 포르피린(porphyrin) 유도체가 sulforaphane와 반대로 HO1을 억제하는
대, 이는 ICH에 의한 조직 손상을 역제한다.\textsuperscript{1,2} 따라서 ICH에
서 HO1의 역할에 대하여 보다 많은 연구가 필요하였다.

산화스트레스와 ICH에 의한 손상

이전에 알려진 바와 같이, 산화스트레스는 ICH 이후의 조직
손상에 매우 중요한 역할을 담당한다. 자유르라디칼과 ICH 손상의
원인적 관계에 대한 직접적 증거는, 항산화제를 치료제로 사용한 실험에서 증명되었다. Dimethyl-thiourea, α-phenyl-
N-tert-butyl nitrite, NXY-059 (α-phenyl-N-tert-
butyl nitrite의 실험유도체) 혹은 deferoxamine, 산화성 칼
분을 길러내는 약물 중 자유르라디칼 청소 물질인 ICH의 동
물 모델에서 녹소증을 유발하게 감소시킨다는 것이 보고된
다.\textsuperscript{1,3,9} 이러한 약물적 실험과 마찬가지로, 유전적 조작을
 통하여 활성 산소종(reactive oxygen species)을 생성하는 다
관주의 약물 paulING 산화 효소가 제거된 마우스는 ICH 이후 조직
손상이 감소됨을 보고하였다.\textsuperscript{10} 최근 에스토로
제네이션 및 체외에서 허혈에 의한 독성을 완화시킨다는 것이
알려졌고, 이는 부분적으로 ICH 이후 기능성 손상에 대한 성별
차이가 칼분 독성을 처리하는 능력의 차이에 기인함 가능성을
시사하고 있다.\textsuperscript{11}

전임시험결과 자유르라디칼을 통한 치료의 가능성을
제시하고 있으나, NXY-059을 이용한 임상시험 결과는 매우
실망스러웠다. NXY-059은 혈관내피증에 효과가 없었을 뿐만
아니라 ICH 환자에서도 도움이 되지 않았다.\textsuperscript{3,10} 이러한 결과의
원인은 불분명하다. 그러나 약물의 약물동역학적 효소(혈관내
피조절을 통과하지 못하였을 가능성) 및 투여된 약물의 활화반응
적(stochiometry)으로 매우 많은 양의 자유르라디칼을 화학식
거지 못하였을 가능성 등이 지적되고 있다.

1. Deferoxamine과 칼분 약독화

Deferoxamine은 이전의 임상시험에서 효과가 있을 것으로
기대되었던 항산화제이다.\textsuperscript{12} 앞서 기술한 바와 같이, 혈관 외 용
혈로 인하여 유리된 자유 칼산의 독성 및 HO에 의하여 매개되
는 대사 과정은 잘 알려져 있다.\textsuperscript{13} 2차 칼산은 H2O2와 반응하여 수
산화 라디칼(hydroxyl radical)을 생성한다. 그러나 deferox-
amine은 칼산과 안정적인 복합체를 형성하기 때문에 유리 칼
산이 화학 반응을 입히기에는 막는다. 그러나 deferox-
amine의 단점으로, 이는 칼산에 의한 산화성 손상 작용을 막
을 뿐만 아니라 프로필산화효소(prolyl hydroxylase, 칼산
을 조소로 사용)의 활성도 저하시켜 HIF의 생체 이용량을 감소시켜 HIF의에 의한 신경보호 효과 감소시킬 가능성이
지적되고 있다. 그러나 deferoxamine은 암모니아 대사에 사용
한 ICH 동물 모델에서 치료 효과가 있는 것으로 보고되었
다.\textsuperscript{14,15} 최근 ICH 환자에서 deferoxamine을 투여하여 가능성
(feasibility), 안전성 및 최대 허용 무게량을 평가하는 제1상
연구가 성공적으로 완료되었다(Magdy Selin, 개인적 교신).

2. 산화성 손상에서 Nrf2의 역할

자유르라디칼 생성 증가와 함께, ICH에 의한 뇌조직 손상 이후
내재적인 항산화효소 시스템의 기능 저하가 발생한다. Hua 등
\textsuperscript{16}은 용혈시킨 적혈구를 뇌에 주입하였는데, 뇌 항산화 방어 시스템
의 가장 핵심적 효소인 manganese superoxide dismutase (manganese superoxide dismutase) 및 구리/아연 과산소디스마타제
(copper/zinc superoxide dismutase) 활성도가 유의하게 감
소함을 보고하였다. ICH가 발생한 뇌에서 항산화 기능이 저하
되어 있을 수 있으며, 따라서 뇌의 내재적 항산화 기능을 촉진
하는 전략을 통하여 치료적 효과를 거둘 수 있을 것이다. 이러
한 가설을 검증하기 위하여, 저자 및 다른 연구자들은 폐쇄위해
계 존재하는 전사요소인 Nrf2를 이용해 보고하였다.\textsuperscript{17,18} 이
는 항산화 반응에서 매우 중요한 역할을 한다. 항산화 반응을
유도하는 요소와 결합함으로서, Nrf2는 과산소디스마타제, 과
산화소 분해효소(catalase), 클루타치온-S-전화효소-1 (glutathione-S-transferase-1), glutathione peroxidase,
HO-1, NAD(P)H quinone oxidoreductase-1, pereoxire-
doxin 혹은 thioredoxin 등 다양한 해독효소 및 항산화효소
발현을 조절한다. ICH 이후 동물 모델에서 Nrf2를 활성화하
기 위하여, 저자들은 자연적으로 존재하는 항산화 유기물 분자인
sulfurphane를 이용하였다. 기대한 결과와 같이, ICH 동물 모
델에 sulfurphane를 투여하였으나 과산화소 분해효소, 과
산소디스마타제, 클루타치온-S-전화효소-1 등 Nrf2에 의하
여 조절되는 항산화 유전자 발현이 효과적으로 증가하였다.\textsuperscript{19}
 이러한 항산화물질의 발현은 ICH가 발생한 뇌조직에서 단백질이
나 지질에 대한 산화성 손상을 감소시키고 신경학적 손실을 감
소할 수 있을 것이다.\textsuperscript{19} ICH의 병태생리 기전에서 Nrf2에 의한
긍정적 효과를 극복적으로 검증하기 위하여, 저자들은 Nrf2가
결손된 마우스(knockout)가 ICH 유도 이후 더 현저한 신경학적
손실을 보임을 확인하였다. 즉, Nrf2는 ICH 이후의 병태생
리 과정에서 산화스트레스에 저항하는 중요한 안전 장치임을
보인 것이다.\textsuperscript{20}

3. PPARγ의 세포보호 효과

Peroxisome proliferator–activated receptor-γ (PPARγ)는 리간드 의존성 전사 요소로서, peroxisome–proliferator response
element라고 불리는 고유한 DNA 서열에 대한 핵 수
용체인 retinoic acid receptor의 이상형체(heterodimer)
로 결합하여 목표 유전자 발현을 조절한다.\textsuperscript{21} 이는 지방과 포
도당 대사 및 에너지 저장 과정에 관련된 유전자 발현을 조절
하는 것으로 잘 알려져 있으며, 또한 PPARγ의 활성화는 항산
화 손상 및 구리/아연 과산소디스마타제 등 과당산의 H2O2을
산소와 물로 분해하여 산화스트레스를 감소시키는 두 개의 효
소 발현을 유도한다.\textsuperscript{41,42} PPARγ의 리간드는 지방산, 비스테로이드 항염증제, 15Deoxy-
Δ\textsubscript{12,14}-Prostaglandin \textsubscript{J2} (15D-
PGE\textsubscript{2}), 반응성의 막 지상 대사 산물과 프로스타글단더의 하위 산물이며, PPARγ의 세레독성 작용북로 생각되고 있다) 및 제2형 당뇨병의 치료제로 사용되는 티아조필드린(thiazolidinedione) 및 피고글리타초네오리그리타초네오리( 합장을 포함한다.\textsuperscript{43} 치료제로 작용주입한 ICH의 림프 모델을 이용한 실험이, 저자는 최근 혈증 내로 15D-PGE\textsubscript{2}를 투여하면 PPARγ가 뇌조직에서 활성화되며 ICH의 발생 뇌내로 과산화소 분해효소의 활성을 주로 신경세포 및 미생세포세포에서 증가함을 보고하였다.\textsuperscript{44} 이러한 생화학적 반응은 신경세포의 사망 감소와 관련이 있으며, 이는 신경세포에서 DNA 절단 및 신경학적 가능 저하의 감소로 증명되었다.\textsuperscript{45} 이러한 발견은 PPARγ가 신경세포 및 다른 세포를 감소하는 손상 및 혈류로부터 보호하는 기능을 한다는 기존의 연구 결과와 일관된다.\textsuperscript{46,47}

염증, 혈액 독성 및 ICH 치료의 목표로써의 혈증제거

뇌의 대식세포인 미세세아세포는 그 수가 상당히 많으며(전체 세포의 10\textasciitilde15\%에 이른다), ICH 이후 수분 내에 즉시 활성화된다.\textsuperscript{48,49} 활성화된 미세세아세포는 염증성 티아토인과 화학구조인지자(chemotactic factor)를 분비하여, 혈액에서 유리하는 염증세포가 ICH에 의하여 손상받은 곳으로 모일 수 있도록 돕는다. 이는 초기에 일시적인 증상을 잠재적으로 나타나며 (18시간\textasciitilde4일), 이후 장기적으로 (1일\textasciitilde수개월) 혈액 유리 대식세포가 뇌조직에서 발견된다.\textsuperscript{48} 염증 신호는 서로 다른 분자와 세포를 잘 조화시키며, 주로 어느 세포나 존재하는 전사인자인 nuclear factor kappa-B (NF-κB)에 의하여 조절된다.\textsuperscript{50}

NF-κB의 목표 유전자는 다양한 결합 분자(intercellular adhesion molecule-1 등 면역 세포의 유출에 관여하는 인자), 시토카인, 케로카인(IL-1β, tumor necrosis factor-α 등 염증성 신호의 전달 및 NF-κB의 활성화에 관여), 급속\textasciitilde단계 분해효소(matrix metalloproteinase-9 등), 면역 수용체, 급속 반응성 단백질(acute phase protein), 세포 표면 수용체 및 염

수용성 효소( inducible nitric oxide synthase, COX-2, PLA\textsubscript{2} 등) 등이다. 자유자유로 역시 NF-κB의 활성화에 중요한 신호 전달 분자로 작용한다. 이러한 NF-κB의 성질은 부분으로 삼화수소가 ICH 이후의 염증 반응을 증가시키는 경향을 설명하고 준다. 실험 연구를 통해 ICH가 발생한 뇌조직에서, ICH 발생 이후 15분 시점부터 활성화되기 시작하며, 1\textasciitilde3일째에 최고에 달하여 이후 수주간 지속되어 보고된 바 있다.\textsuperscript{51} NF-κB와 의하여 조절되는 목표 유전자들인 IL-1β, tumor necrosis factor-α, matrix metalloproteinase-9 등 ICH에 의한 뇌조직 손상 과정에 기여하는 요소임을 기억하는 것이 중요하다.\textsuperscript{52\textendash53}

미세세아세포 및 대식세포에 의한 염증 반응이 ICH 이후 뇌조직 손상을 악화시키며, 미세세아세포 및 대식세포에 의한 세포 대식 과정은 이후 뇌의 추출 과정에 기여하기도 하며, 이후 조절 및 가능적 회복 과정에 기여하는 중요한 역할이 있다.\textsuperscript{54} 세포사멸 및 전위된 세포(유출된 적혈구 등) 및 손상을 입은 세포가 염증 신호를 보내내기 전에 빠르고 효율적으로 제거하는 것 같은 이상적인 손상을 줄이는 데 중요한 것이다. 미세세아세포 및 대식세포는 다양한 세포 표면 유전자를 발현하며, 이들 중 하나인 초소 수용체(scavenger receptor: CD\textsubscript{91}, scavenger receptor-α, MARCO, scavenger receptor-B\textsubscript{1} 및 CD\textsubscript{36} 등)는 대식 혹은 세포 내 괴합 과정을 통해 ICH 이후의 조직 손상 과정에서 발생한 세포 관리에 기여함을 것으로 보인다.\textsuperscript{55} 연구를 통해 ICH상 청소 수용체이며 PPARγ에 의하여 전사 조절되는 CD\textsubscript{36}을 분석한 연구는 세포 및 체외 모델을 통해 (1) 미세세아세포/대식세포는 CD\textsubscript{36}을 이용하여 적혈구의 대식을 촉진하고 (2) PPARγ 작용체(rosiglitazone, pioglitazone 혹은 15D-PGE\textsubscript{2})를 투여하면 CD\textsubscript{36}이 활성화하여 보다 빠른 혈중 흡수 및 ICH 이후 기능적 회복 향상을 기대할 수 있음을 보고하였다.\textsuperscript{56} 이 연구에서 가장 흥미로운 결과는, PPARγ 효능체를 통한 제거 및 체외 실험에서 대식 과정을 촉진한 결과, 이후 염증 반응(1L-1β, tumor necrosis factor-α, matrix metalloproteinase-9 mRNA 감소)이 감소하였다는 점이다.\textsuperscript{57} PPARγ의 작용 기전 중 하나가 NF-κB의 DNA 결합을 억제하는 것이기 때문에, PPARγ의 항염증 특성을 이용한 NF-κB의 기능 억제를 통한 것으로 생각되고 있다.\textsuperscript{60} PPARγ를 이용한 염증반응질의 긴장된 결과 덕분에, ICH 혈중 흡수에 있어 pioglitazone의 안전성을 평가하는 임상시험이 2009년에 시작되었다(http://clinicaltrials.gov).
Sources of Funding
Supported in part by National Institute of Health, National Institute of Neurological Disorders and Stroke (RO1NS060768, R21NS057284, and R01NS064109).

Disclosures
None.

References


