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392 *Current Neuropharmacology*, **2016**, *14*, **392-402**

Molecular, Cellular and Clinical Aspects of Intracerebral Hemorrhage: Are the Enemies Within?

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Abstract: Hemorrhagic stroke is a disease with high incidence and mortality rates. In addition to the mass lesions that result from hemorrhagic stroke, substances such as the blood-derived products (BDP) (hemoglobin (Hb), heme and iron) induce a potent inflammatory response and exert direct toxic effects on neurons, astrocytes, and microglia. In the present review, we discuss the mechanisms of brain injury secondary to hemorrhagic stroke, focusing on the involvement of BDP as major players of cellular redox imbalance, inflammation, and glutamate excitotoxicity. Potential natural mechanisms of protection against free Hb and heme such as haptoglobin and hemopexin, respectively, are highlighted. We finally discuss the experimental and clinical trials targeting free iron and heme scavenging as well as inflammation, as potential new therapies to minimize the devastating effects of hemorrhagic stroke on brain structure and function.

Keywords: Heme, hemorrhagic stroke, inflammatory response, intracranial bleeding, iron, reactive oxygen species, subarachnoid hemorrhage.

INTRODUCTION

Each year, 795,000 people have strokes in the United States, or 1 person every 40 seconds, on average. These incidents account for approximately 103,000 cases of hemorrhagic stroke each year in the United States and 2 million cases worldwide [1]. Intracerebral hemorrhage (ICH) is a deadly disease, with an estimated mortality rate of approximately 40% within one month following an event, and only 12-39% of the survivors can perform activities of daily living at the time of hospital discharge [1]. Ten years following the first stroke, only approximately 24% of patients will still be alive [2]. In the coming years, ICH cases are expected to grow, due to the aging of the population and the increasing use of anticoagulants and thrombolytics, which currently accounts for 20% of brain hemorrhage cases in the United States [3]. Thus, understanding the pathophysiology of brain injury after ICH is of pivotal importance for developing new therapeutic approaches that can reduce these high morbidity and mortality rates.

The pathophysiology of ICH is very complex and begins with a massive release of blood within the brain parenchyma, after which red blood cell (RBC) lysis begins almost immediately, releasing hemoglobin (Hb), heme, and iron into the central nervous system (CNS). Increased plasmatic levels of heme, as well as decrease in the content of the major heme binding protein in plasma, hemopexin (Hx), have already been associated with increased mortality in severe sepsis [4], and to the severe systemic manifestations of malaria [5]. Also, the involvement of iron as a major component in neurodegenerative diseases, such as Alzheimer and Parkinson's [6] has been increasingly demonstrated. In brain hemorrhage, many experimental studies have clarified the toxic effects of heme and iron upon brain tissue and some pre-clinical trials have investigated the potential effect of iron chelation, increased antioxidant defenses, and anti-inflammatory therapies as potential new approaches to minimizing the devastating outcome of hemorrhagic stroke. In the present manuscript, we review the mechanisms of ICH brain injury secondary to BDP release within brain parenchyma, the protective systems against blood-derived brain injury, as well as the experimental studies and clinical trialswhich investigated the reduction of BDP-related toxicity to the CNS.

MECHANISMS OF BRAIN INJURY

The Blood-Derived Products (BDP)

Some studies have implicated blood-derived products (BDP) such as Hb, heme, and iron as key mediators of brain injury. Erythrocyte lysis occurs within minutes and continues for several days following hematoma formation, releasing BDP into the brain parenchyma [7-9]. Once in the extracellular

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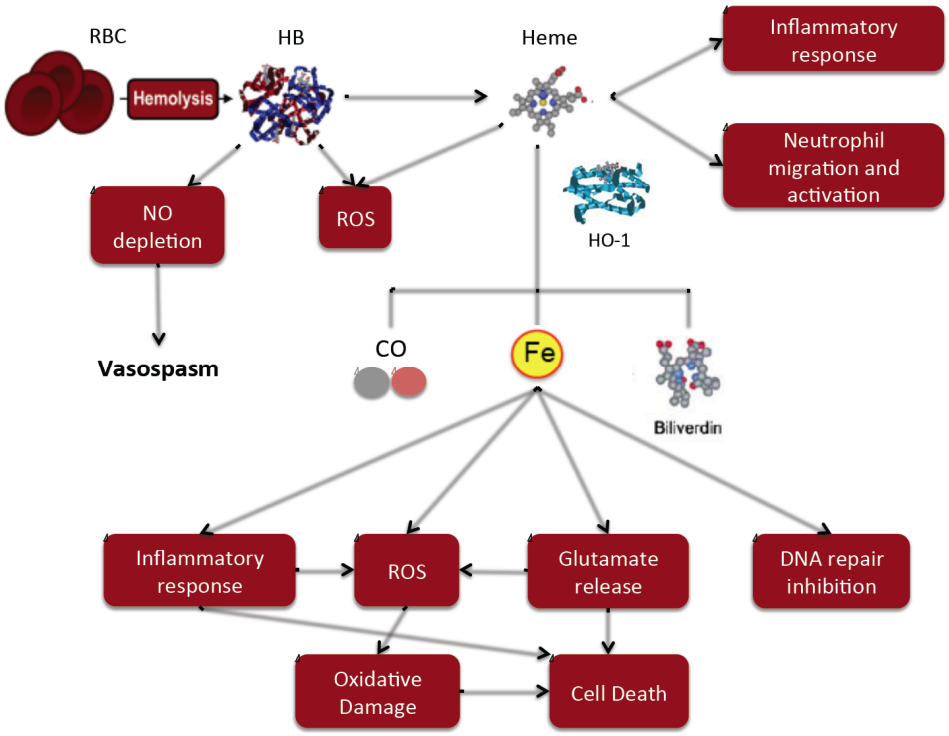

Fig. (1). Schematic summary of the main mechanisms of brain injury after hemorrhagic stroke. Hemoglobin and its metabolites can exert deleterious effects on brain tissue through NO depletion and vasospasm, ROS production, stimulation of inflammatory response, inhibition of DNA repair, and glutamate release. (Adapted from Larsen R *et al*.) [12].

milieu, Hb is eventually digested by still poorly understood mechanisms, releasing heme, which is further degraded into biliverdin, carbon monoxide, and iron by heme oxygenases (HO). Hb, heme and iron are potent cytotoxic BDP [10] that, through a wide array of mechanisms, boost the inflammatory response [10] and promote protein, nucleic acid, carbohydrate, and lipid oxidation, disrupting cell signaling and causing ultimately cell death. The myriad of cellular effects caused by BDP, and their consequences, are briefly summarized in Fig. **1** [11, 12, 14].

Several lines of evidence converge to the fact that BDP play a central role on the pathogenesis of ICH-associated injury. For example, infusion of packed erythrocytes induced edema and caused neurological deficits several days following injury, which suggests a role for erythrocyte lysis in delayed brain damage [13]. In line with these evidences, infusion of lysed erythrocytes results in brain edema, blood-brain barrier (BBB) disruption, and DNA injury as early as 24 h, strengthening the concept that BDP exert toxic effects in the CNS [14, 15]. Indeed, free Hb is not only a pro-oxidant molecule, generating highly reactive hydroxyl radicals in an ascorbate-dependent manner [16], but also cause CNS damage [17]. Hb is also able to interact with nitric oxide (NO) [18], which cause Hb iron oxidation [19] and NO decomposition into nitrate (NO_3^-) [20]. NO consumption by free Hb not only mediates vascular hypertension [21] but also seems to be directly involved in vasoconstriction in subarachnoid hemorrhage [22, 23].

The pro-oxidant environment of the hemorrhagic brain allows Hb oxidation to metHb, which contributes to heme release, protein carboxylation, LDL oxidation [24], and tissue injury [25]. Another interesting aspect is that neurons and astrocytes are morphologically, functionally and metabolically distinct cells, which exhibit different susceptibilities to BDP [27-31]. Noteworthy, neurons are remarkably more susceptible to Hb and heme, when compared to astrocytes, in a mechanism involving increased uptake of these BDP and redox imbalance in neurons [27]. On the other hand, the HO-1 isoform plays a central protective role in astrocytes against Hb toxicity [26, 32] whereas HO-2 activity in neurons contributes to Hb-related neurotoxic effects [28].

Iron is an essential cellular element and isutilized in a wide array of biochemical reactions in the CNS, such as neurotransmitter metabolism, myelin synthesis, and in cellular energy transduction reactions. Concentrations of brain iron are highest at birth, decrease during the first two weeks of life, subsequently increasing along life [27, 28], suggesting that the brain capacity deal with iron overload decreases with age. Iron toxicity to the CNS is mediated by various mechanisms, the most important one through redox imbalance, due to its capacity to generate hydroxyl radical by the classical Fenton reaction [35] as well as to promote metal-catalyzed oxidation [36]. In this scenario, it must be emphasized that brain is highly susceptible to redox imbalance, as a tissue enriched with unsaturated fatty acids, iron, and ascorbate, with a high oxygen demand per mass [37] which contrast with strikingly low activities of antioxidant defenses, especially catalase in different brain regions [37]. Thus, after ICH, brain antioxidant defenses are consumed to prevent redox imbalance and ultimately tissue damage. Sadrzadeh and colleagues have shown that iron and Hb catalyzed hydroxyl radical production and lipid peroxidation

[16, 17, 29]. High levels of protein carbonyl were detected in the perihematomal white matter within minutes following autologous blood injection [30]. In fact, redox imbalance can persist for up to three days following injury, as demonstrated by the increased dihydroethidium staining (a marker for redox imbalance detected *in situ*) in the peri-ICH region [31]. Reductions in superoxide dismutase (SOD) activity and increased DNA fragmentation following ICH were also described [15]. In addition to their role in direct injury to cell membranes, ROS can activate the transcription factors NF-kβ [32] and activator protein-1 and can also induce BBB disruption, worsening the brain edema [33]. ROS are also able to modulate mitochondrial function, reducing respiratory rates by interfering with electron transport system (ETS) activity [34, 44]. Iron can further propagate oxidative injury by inhibiting the enzymatic function of base excision repair pathway for DNA damage and by delaying the repair of DNA in cultured neurons [35].

To date, there has been only one clinical study that has examined ROS production as a mediator of brain injury following ICH. Mantle and colleagues found oxidized proteins in perihematomal brain tissue samples following hematoma drainage in 10 patients [36]. However, evidence of ROS production was also found in the control samples (patients submitted to brain tumor resection or aneurysm clipping). It was hypothesized that the control patients were also subjected to higher levels of oxidative stress due to their underlying pathology (brain cancer and intracranial aneurysms). Despite the lack of clinical evidence, abundant experimental data show that ROS generation is a key component of brain injury following ICH [47, 48].

Another mechanism of brain injury by iron is by the amplifying of the inflammatory response. Lipopolysaccharide (LPS)- activated microglia loaded with iron had increased the release of MMP-9 [37], TNF-α, and IL-1β than non-loaded microglia [38]. Culture media from activated microglia was toxic for oligodendrocytes, an effect that was reversed by iron chelation [38]. Consistently, increases in iron levels also led to activation of NF-κβ [38].

Glutamate excitotoxicity seems to be an important mechanism in neuronal and oligodendrocyte cytotoxicity mediated by iron. The evidence accumulated so far indicate that glutamate promotes iron uptake in rat spinal cord explants [39], increase BBB permeability, and promote brain edema through NMDA receptors, which are stimulated by redox imbalance and inhibited by iron chelation [40-42]. Curiously, iron mediate glutamate toxicity by stimulating glutamate release and aconitase activity [43], which are important for both glutamate synthesis, and energy metabolism. Furthermore, increasing evidence suggests that iron can induce neurodegeneration, promote neuronal autophagy [44], enhance β-amyloid neurotoxicity through transglutaminase expression [45], and cause neuronal atrophy and death [46]. In humans, serum ferritin [47] and hematoma iron content [48], as measured by magnetic resonance imaging (MRI), were associated with perihematomal edema development.

Hb and heme can also directly contribute to brain injury. Heme is the prosthetic group of many different heme proteins and contains a central iron atom coordinated to the protoporphyrin ring. Although RBC lysis and heme degradation are well-studied processes in ICH, the mechanisms governing Hb degradation are far less understood, and it seems that heme release upon RBC disruption would be explained by Hb oxidation to metHb during ICH [61]. Also, thrombin was also implicated in this process [62] but whether this protease mediates Hb degradation and subsequently heme release remains elusive. Regardless the exact mechanism of Hb digestion, once heme is released from globin polypeptide chain, it can act as a potent cytotoxic pro-oxidant compound and lead to oxidative stress [49]. "Free" heme can also oxidize some circulating components such as LDL particles, which exert cytotoxic effects on endothelial cells [24]. These observations are supported by the use of pharmacological antioxidants which confer cytoprotection against "free" heme [50].

In addition to direct stimulating oxidative stress, heme also participates in the inflammatory reaction by directly stimulating TLR4 [11, 15] or amplifying the inflammatory effects of microbial molecules [52] as can be seen in this paper from Figueiredo *et al*. [10]. Fig. **2** summarizes the effects of heme upon TLR4. Heme can also induce neutrophil migration, decomposition of organic radicals into highly reactive alkoxyl and peroxyl radicals [53], and secretion of IL-8 [54] and TNF-α[66]. Furthermore, heme induces not only the expression of pro-inflammatory adhesion molecules, both *in vitro* [55] and *in vivo* [56], but also vascular permeability [54], events that contribute to brain edema. Besides promoting inflammatory reaction within the CNS, heme was also found to induce programmed cell necrosis in macrophages *in vivo* [36]. Furthermore, neurons were found to be more sensitive to the toxic effects of heme [57] and Hb [26] than astrocytes, and the cell death further propagates brain injury. Interestingly, recent studies demonstrated a critical role of TLR4 in the pathogenesis of hemolytic and hemorrhagic conditions [58].

MECHANISMS OF BRAIN PROTECTION AGAINST BDP TOXICITY

In the setting of severe hemolysis, several protective mechanisms are activated reducing the deleterious effects of free iron, heme, and Hb. The main protective mechanisms consist on heme degradation by the heme-oxygenases into iron, carbon monoxide, and biliverdin, intracellular iron sequestration by ferritin [74, 75], as well as Hb and heme scavenging by haptoglobin (Hb) and hemopexin (Hx), respectively [76, 77]. While the haptoglobin and hemopexin-based defense mechanisms are well described in hemolytic diseases, such as malaria and other hemolytic anemias, their role in brain protection after hemorrhagic stroke is less clear. Compounds that up-regulate the expression of antioxidants, like Nrf2 and PPAR-γ, also play a role in cerebral protection after intraparenchymal bleeding.

Haptoglobin and Hemopexin

Haptoglobin (Hp) and hemopexin (Hx) are plasma proteins that are synthesized in the liver, and their major functions described so far are to bind circulating Hb and heme, respectively, that have been released during intra-vascular hemolysis and to remove them from circulation.

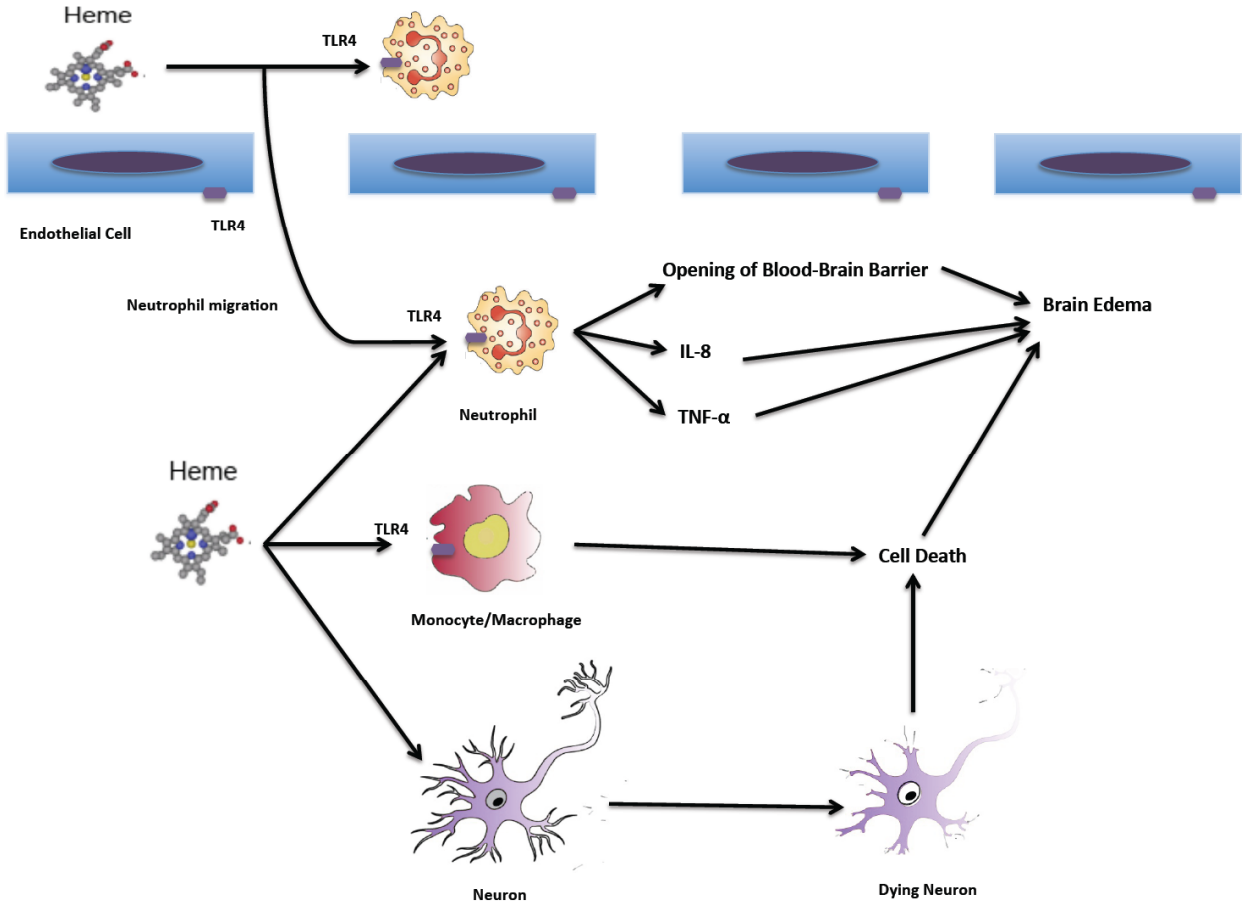

Fig. (2). Schematic summary of TLR4 activation by heme and its effects. Heme induces neutrophil migration across the blood-brain barrier and secretion of IL-8 and TNF-α by stimulating TLR4. The stimulation of inflammatory response and augmentation of vascular permeability contribute to brain edema. Besides promoting inflammatory reaction within the CNS, heme also induces programmed cell necrosis in monocytes/macrophages. Furthermore, the uptake of heme by neurons induces cell death, which further propagates the inflammatory response.

Haptoglobin-Hb complexes are uptaken by macrophages/ microglia through the scavenger receptor CD163. Recent evidence suggests that Hp and Hx may play roles in Hb and heme scavenging in the CNS following ICH. In this sense, Zhao and colleagues have shown that Hp expression is increased in the perihematomal area following ICH [59]. In addition to Hp transport to the brain parenchyma as a result of BBB disruption, Hp can be synthesized by oligodendrocytes, which was demonstrated in neuron-glial co-culture experiments [59]. Furthermore, oligodendrocytes protect neurons from Hb toxicity through Hp release, and hypohaptoglobinemic mice experienced more extensive brain damage, neurological deficits, neuronal loss, and white matter injury following ICH compared to controls [59]. These results suggest that Hp may be an important component of CNS protection by Hb chelation. However, Galea and colleagues reported that most Hb was not bound to Hp, which suggests that the CD163-Hb-Hp system is saturated and that the primary route for Hb clearance from the CNS is freely crossing the BBB through a concentration gradient [60]. Moreover, hypohaptoglobinorrhachia patients, which exhibit more effective clearance of Hb, have been associated with a reduced incidence of delayed cerebral infarct (DCI) [60]. This evidence suggests that, although Hp secretion is a protective mechanism against free Hb, it may not be considered as a main mechanism of protection in the brain. The main components of brain protection against blood extravasation and drugs tested to enhance the mechanisms of protection are summarized in Fig. **3**.

Hx is a plasmatic glycoprotein that is synthesized by hepatocytes playing a central role in heme scavenging. Hx binds to heme and forms a stable heme-Hx complex, which is cleared by CD91 macrophages [61]. In the human brain, Hx synthesis occurs primarily in neurons (80), but not in oligodendrocytes, and is induced by heme [80, 81]. The formation of heme-Hx complexes may facilitate heme removal by microglia/macrophages following ICH. Data supporting this hypothesis, however, is scarce. In Hx knockout mice, the striatal cell viability three days following injury was significantly reduced, heme tissue content was 2.7-fold increased, and locomotor activity was reduced compared to wild-type mice [62]. Deletion of Hx resulted in increased infarct volumes and neurological deficits [63]. Moreover, heme-Hx complexes protected neurons from oxidative stress-associated cell death and induced the expression of heme-oxygenase 1 (HO-1) [64]. Hx also

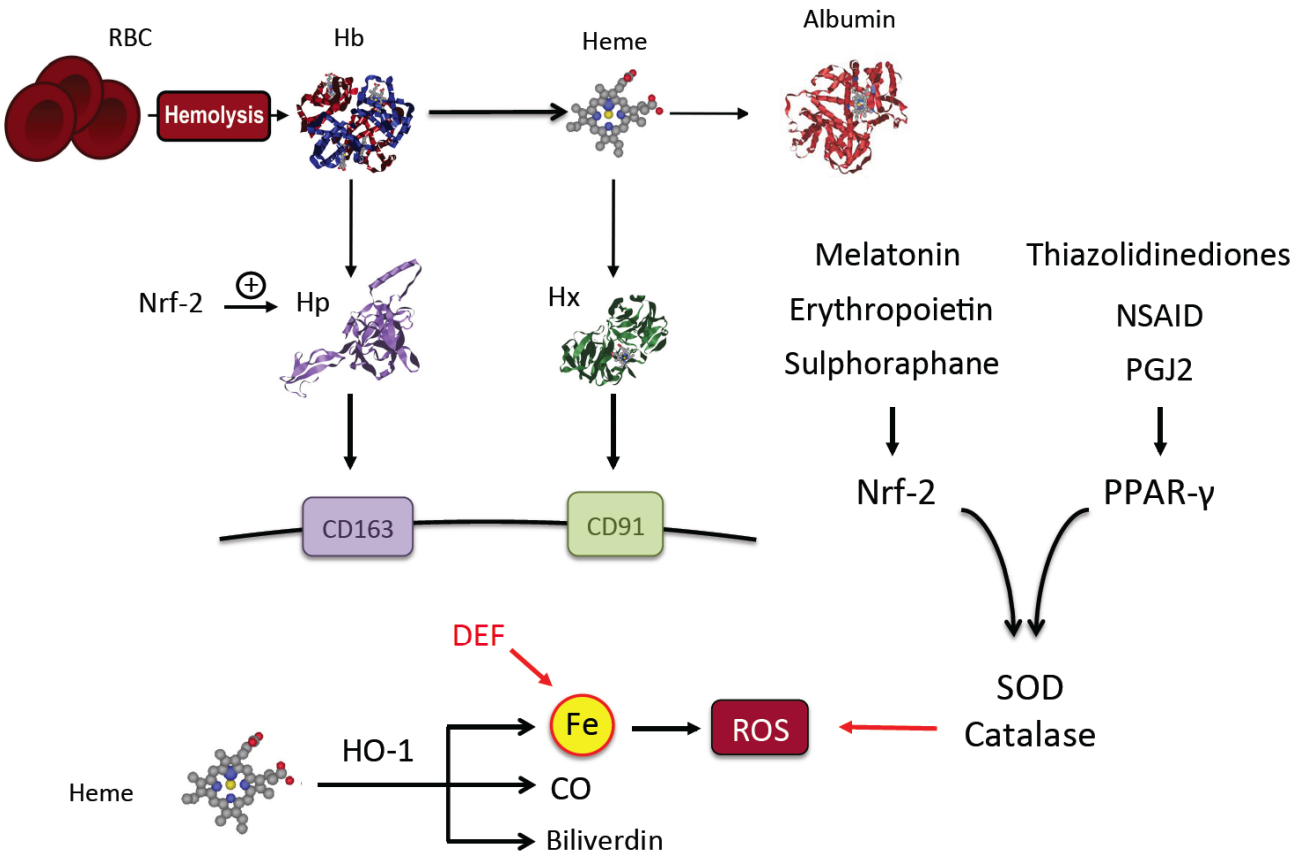

Fig. (3). Schematic mechanism of brain protection and pharmacological compounds tested in experimental and clinical trials. Many drugs tested to this date act either by iron scavenging or by enhancing antioxidant response after hemorrhagic stroke (adapted from Larsen R, *et al.*) [12].

decreased intra-neuronal heme accumulation and decreased heme breakdown [64]. Therefore, while Hp and Hx are well-established protective mechanisms after systemic hemolysis, evidence for their role in protecting the brain after ICH is lacking.

Heme Oxygenase

Heme oxygenase (HO) is a rate-limiting enzyme of physiological heme degradation that catalyzes the conversion of heme to biliverdin, carbon monoxide, and iron. There are two known isoforms of HO (HO-1 and HO-2), being HO-2 constitutively expressed and is found in most cell types (including neurons), whereas HO-1 is induced following ICH in microglia/macrophages [63, 65]. HO-1 expression reaches its peak at 3 and seven days following brain hemorrhage [66]. In an autopsy study, HO-1 expression began approximately two h following ICH, peaked within 17-30 h and declined after ten days [67].

The role of HO activity in ICH is controversial. Genetic deletion of HO-2 led to neurons that were more vulnerable to heme toxicity. Mice with genetic deletion of HO-2 had a 30% increase in brain volume injury on the first day after intracerebral hemorrhage and a 67% increase on the third day, as well as worsened neurological function on the first and third days after ICH [65]. HO-2 knockout mice were more susceptible to brain damage following ICH, had increased neutrophil infiltration, microglial/macrophage and astrocyte activation, DNA damage, peroxynitrite production, and cytochrome c immunoreactivity [83].

On the other hand, HO-1 null mice exhibited reduced brain injury, neurological dysfunction, leukocyte infiltration and microglial activation, as well as reduced susceptibility to DNA damage [87]. In animal models of Alzheimer and Parkinson's diseases, HO-1 expression promoted intracellular oxidative stress, the opening of the mitochondrial permeability transition pore, and the accumulation of non-transferrin iron in the mitochondrial compartment [68, 69]. In line with these observations, HO-1 knockout astrocytes demonstrated a 20-25% death rate and a fourfold increase in protein oxidation [70]. However, Chen and colleagues have described that the increased sensitivity to heme toxicity observed in HO-2 knockout mice was reduced when HO-1 expression was stimulated by adenoviral gene transfer [71]. The prevention of heme accumulation at intracellular toxic levels and the production of the antioxidants biliverdin/bilirubin may partially explain this protection [69, 72]. As a result of HO-1 activity, carbon monoxide is produced, which play a protective role in a rat model of ICH [73]. Thus, when a transient rise of intracellular "free" heme takes place, the HO-1 expression is enhanced, degrading this molecule into free iron. From the data exposed above, it can be concluded that HO-1 has both deleterious and protective properties in ICH. The early up-regulation of HO-1 possibly fit with the events and is protective against oxidative stress, whereas its

overexpression may result in its dysfunction and promote further toxicity.

Nrf2 and Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ)

Nrf2 is a transcriptional factor that promotes transcription of antioxidant genes, including quinine oxidoreductase 1, glutathione S-transferase, glutamate-cysteine ligase, glutathione peroxidase, and HO-1 [74]. Nrf2 is present in neurons, astrocytes, and microglia and is regarded as being neuroprotective. Shah et colleagues demonstrated that Nrf2-/- mice were more prone to stroke damage than control mice following ischemia-reperfusion injury and that tert-butylhydroquinone, a Nrf2 inducer, attenuated neuronal death [75]. In a collagenase model of ICH, Nrf2-deficient mice were more susceptible to severe neurological deficits, and worsening of brain injury was associated with increases in leukocyte infiltration, ROS production, DNA damage, and cytochrome c release [76, 77]. Also, Nrf2 depletion increased the inflammatory reaction through the NF-κB pathway, stimulating the expression of TNF-α, IL-1β, IL-6, and MMP-9 [78]. Melatonin and erythropoietin appear to protect against early brain injury by stimulating the Nrf2-pathway [79, 80]. These studies suggest that Nrf2 expression is neuroprotective against early inflammatory brain injury in hemorrhagic stroke models.

PPAR-γ is another transcriptional factor that regulates the expression of two important antioxidant genes: catalase and superoxide dismutase. Zhao and colleagues have shown that the intrahemorrhage injection of 15D-PGJ_2 leads to the increased expression of PPAR-γ and catalase in neurons and microglia [81, 82]. PPAR-γ also reduced the expression of the pro-inflammatory genes TNF-α, IL-1B, MMP-9, and iNOS, the extracellular H_2O_2 levels, and prevented neuronal damage. These effects were parallel to stimulation of phagocytosis by microglia [81, 82]. PPAR-γ has also been associated with reduced neurological dysfunction and hematoma resolution [81, 82]. Supporting these evidence, rosiglitazone, an agonist of PPAR-γ, attenuated MPO activity and the expression of IL-1β and TNF-α [83]. This compound also reduced oxyHb-induced TLR4 expression and TNF-α release in a culture of vascular smooth muscle cells [84], as well as reduced cerebral vasospasm following SAH by impairing TLR4 signaling [85]. In summary, PPAR-γ seems to exert a protective role after hemorrhagic stroke by reducing the inflammatory response and by increasing antioxidant protection. Thus, PPAR-γ agonists rise as potential drugs for clinical trials.

EXPERIMENTAL AND CLINICAL TRIALS

Based upon evidence of iron and heme-induced brain injury and the protection mechanisms, many experimental and clinical trials have focused on three primary targets to reduce iron-mediated neuronal injury: iron scavenging, inhibition of inflammatory reaction induced by iron and heme, and the enhancement of natural protection pathways, such as increasing expression of haptoglobin and hemopexin.

As a transition metal, iron is chemically defined by bearing incomplete electron orbitals, which is an essential feature of any free radical. This mean that as a free radical, iron present in the most abundant biological oxidation forms ($^{+2}$ and $^{+3}$) are naturally unstable species, which may react with nearest molecules to reach their chemical stability. Deferoxamine is an iron chelator that has been used in clinical practice for many years, and it can bind iron in both free and protein-bound forms, with high affinity, forming stable less reactive complexes. As a result, iron chelation by deferoxamine prevents cellular redox imbalance and neuronal death [86, 87]. Deferoxamine also reduced Hb-induced DNA damage, hippocampal neuronal death, brain atrophy and swelling [88]. Beyond these effects, deferoxamine has also been shown to reduce HO-1 expression in aged rats [89], attenuate the accumulation of 8-OHdG, a marker of nucleic acid oxidation, and enhance the secretion of APE/Ref-1, a DNA repair mechanism for oxidative damage [90]. In a recent meta-analysis of pre-clinical trials, deferoxamine was found to improve neurobehavioral scores at the last point of assessment and to reduce brain edema in ICH [91]. However, further studies were unable to find an association between deferoxamine administration and improved neurological outcomes in rat models [92, 93]. Both studies that found negative results for deferoxamine were performed in collagenase-induced rat models of brain hemorrhage, raising questions regarding differences among the different models of ICH and their ability to accurately reproduce clinical practices.

Recently, the safety and tolerability of deferoxamine in ICH patients were investigated. Twenty patients were enrolled, and doses between 7 mg/Kg and 62 mg/Kg per day were tested. The primary side effect was mild hypotension, and it was concluded that deferoxamine was well tolerated and safe in the clinical setting [94]. Further studies to establish the potential role of deferoxamine in preventing neurological dysfunction following ICH are eagerly awaited.

Minocycline is a tetracycline antibiotic, which has been shown to have neuroprotective properties in addition to its antibiotic, anti-inflammatory, anti-apoptotic, and antioxidant effects, and seems to play a beneficial role following the ICH [95, 96]. Power et colleagues. reported that minocycline infusion inhibited IL-1α and MMP-12, diminished microglial activation and neutrophil infiltration in the brain, reduced the appearance of apoptotic cells, and improved neurobehavioral outcomes in a mouse model [97]. Another study showed that the systemic administration of minocycline reduced perihematomal brain edema, neurological deficits, and brain atrophy [95]. However, other investigators have reported that, while minocycline had beneficial effects in reducing brain edema, microvessel loss, neutrophil infiltration, and TNF-α and MMP-12 secretion [96], it had no protective effects on striatal tissue and neuron loss. Xue and colleagues, in an autologous blood mice model, suggested that a high dose of locally applied minocycline with intravenous supplementation might result in better neuronal protection following ICH [98]. In a recent meta-analysis, minocycline was found to improve neurobehavioral outcomes only when infused for at least 24 hours following ICH [91]. All this data together suggest that minocycline may have a beneficial effect after hemorrhagic stroke mainly by reducing the inflammatory response. However, the use of minocycline to minimize the onset and progression of neurodegenerative

diseases has been questioned. A phase III trial with 412 amyotrophic lateral sclerosis patients showed that minocycline was associated with non-significant trends of a faster decline in functional scores and higher mortality rates [99]. In models of Parkinson's and Huntington's diseases, minocycline was also found to be associated with worst functional scores and increased neuronal loss [100]. These trials pose serious questions over the effectiveness of minocycline in the treatment of neurological diseases since experimental trials cannot be directly translated into clinical trials regarding dosing and treatment schedules, and patients are usually not pre-treated before their symptoms arise.

Statins are inhibitors of hydroxymethylglutaryl-CoA reductase, and their primary effect is to reduce cholesterol biosynthesis and, therefore, to diminish blood cholesterol levels. However, in addition to their hypocholesterolemic effect, statins have pleiotropic properties [101], including improving endothelial function, attenuating vascular and myocardial remodeling, and inhibiting vascular inflammation and oxidation. These pleiotropic effects are being studied in a wide variety of diseases, including ICH. In a rat model, atorvastatin administration 24 hours following ICH reduced cell loss in the striatum and improved neurobehavioral outcomes [102]. These effects were attributed to increased synaptic plasticity, decreased expression of iNOS, and neutrophil/microglia recruitment, leading to a reduced inflammatory reaction [103]. Statin was found to reduce brain water content, block neuron apoptosis, and reduce the plasmatic level of MMP-9 [104]. Simvastatin reduced cognitive dysfunction [105] and diminished IL-1β secretion while enhancing TGF-β release and TGF-β positive lymphocyte infiltration in the subarachnoid and perivascular spaces [106]. Therefore, it was hypothesized that a statin-induced Th2 shift could provide neuroprotection. In a mouse model, simvastatin was also found to recouple eNOS and, therefore, prevent eNOS monomer formation, decrease superoxide radical production, and increase NO production, leading to decreased vasospasm and neuronal injury [22].

Despite the beneficial effects observed in animal models, clinical studies have shown conflicting results regarding the association between prior statin use and mortality rates. Naval and colleagues demonstrated that prior statin use was associated with decreased perihematomal edema and mortality rates following ICH [107, 108]. However, FitzMaurice and colleagues found no association between statins and neurological outcomes or mortality rates [109]. More recently, a meta-analysis of more than 2,000 patients with ICH demonstrated an association between prior statin use and good outcomes, as well as reduced mortality rates [110]. On the other hand, an analysis of a Canadian registry of 2,466 patients found no association between preadmission statin use and outcomes in ICH [111], which makes the effects of prior statin use unclear. In the face of the conflicting evidence provided by retrospective studies, clinical trials evaluating the effect of statin administration after hemorrhagic stroke were proposed. Unfortunately, the recently published STASH trial found no difference in short-term and long-term outcomes among patients with subarachnoid hemorrhage receiving either simvastatin or placebo [112].

Sulphoraphane, a known Nrf2 activator, reduced oxidative damage and neurological deficits in animals [76]. Microglia and macrophages microglia/macrophages utilize a surface receptor, CD36, to promote phagocytosis of red blood cell. One specific study evaluated the effect of treating animals with PPAR-γ agonists (e.g., rosiglitazone, pioglitazone, or 15D-PGJ2), which increased CD36 expression, and found out that PPAR-γ agonist treatment results in faster hematoma resolution and improved functional recovery after ICH [113]. 15D-PGJ_2 also increased the expression of catalase, primarily in neurons and microglia, following ICH [81, 82]. Other PPAR-γ agonists that are already in clinical use include the thiazolidinediones, specifically pioglitazone and rosiglitazone, and a clinical trial of pioglitazone in ICH patients is current ongoing [114].

CONCLUSIONS

Brain injury following ICH is a complex phenomenon that involves systemic and local inflammatory reactions, direct toxicity of BDP, redox imbalance and ultimately, cell death. A deeper insight into the mechanisms involved in ICH is required, as well as the development of new therapeutic approaches aiming the morbidity and mortality rates reduction of this deadly disease.

AUTHOR'S CONTRIBUTIONS

CRS designed and drafted the manuscript; MTB and MFO helped draft the manuscript and revised it critically; FAB conceived the review, helped draft the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

LIST OF ABBREVIATIONS

15D-PGJ_2	=	15-Deoxy-Delta-12, 14-prostaglandin J2
BBB	=	blood brain barrier
BDP	=	Blood-derived products
CNS	=	central nervous system
DCI	=	delayed cerebral infarct
H_2O_2	=	Hydrogen Peroxide
Hb	=	Hemoglobin
HO	=	heme oxygenase
Hp	=	haptoglobin
Hx	=	hemopexin
ICH	=	intracerebral hemorrhage
IL	=	interleukin
LPS	=	Lipopolysaccharide

MMP	=	Matrix metallopeptidase
MPO	=	myeloperoxidase
MRI	=	magnetic resonance imaging
NF-κβ	=	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	=	N-methyl-D-aspartate receptor
NO	=	nitric oxide
Nrf2	=	Nuclear factor-like 2
PPAR-γ	=	Peroxisome proliferator-activated receptor gamma
RBC	=	red blood cells
ROS	=	reactive oxygen species
SAH	=	subarachnoid hemorrhage
TGF-β	=	Transforming Growth Factor-β
TLR4	=	toll-like receptor 4
TNF-α	=	tumor necrosis factor alpha

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Received: January 28, 2015 Revised: November 28, 2015 Accepted: December 29, 2015