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Intracranial Hemorrhage – Mechanisms of Secondary Brain Injury

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SUMMARY

ICH is a disease with high rates of mortality and morbidity, with a substantial public health impact. Spontaneous ICH (sICH) has been extensively studied and a large body of data has been accumulated on its pathophysiology. However, the literature on traumatic ICH (tICH) is more limited, and there is a need for further investigations of this important topic. This review will highlight some of the cellular pathways in ICH with an emphasis on the mechanisms of secondary injury due to heme toxicity and to events in the coagulation process, which are common to both sICH and tICH.

Keywords

Intracranial hemorrhage; heme toxicity; iron toxicity; coagulation; inflammation; vascular response

BACKGROUND

ICH is a disease with high rates of mortality and morbidity, with a substantial public health impact. ICH can be classified as spontaneous or traumatic. Spontaneous ICH (sICH) has been extensively studied and a large body of data has been accumulated on its pathophysiology. However, the literature on traumatic ICH (tICH) is more limited. The need to investigate the specific mechanisms of tICH is underscored by the fact that ICH is a well known feature of severe TBI, and carries a high risk of morbidity and mortality. Progression of the hemorrhage is associated with poor clinical outcomes [1, 2]. This is true not only of large hemorrhages, but also of micro-bleeds detected only on susceptibility-weighted imaging (SWI) imaging and not on routine CT or MRI [3]. Moreover, these detrimental sequelae often extend beyond the area of the hemorrhage. Metabolic changes have been found in regions remote from focal hemorrhagic lesions, suggesting diffuse injury after human traumatic brain injury [4]. In a rat TBI model, severity of intracerebral hemorrhage correlates with degree of final cortical atrophy [5]. In addition, TBI itself may induce coagulopathy, which further increases the extent of intracerebral hemorrhage and the incidence of poor outcome associated with such injuries [6].

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The management of traumatic intracerebral hemorrhage (tICH) presents a paradox. On one hand, current management for severe TBI is directed towards preservation of adequate cerebral perfusion pressure (CPP). This approach frequently requires therapies that raise the arterial blood pressure when increased intracranial pressure (ICP) does not respond to efforts to return it to normal levels. On the other hand, increasing the blood pressure in traumatic injuries will likely increase blood loss. Since the progression of the hemorrhage is greatest in the first 24 hours, while the edema formation begins immediately after trauma and commonly peaks within 48-72 hours, the current CPP-driven management may be detrimental in terms of ICH progression. Ideally, the management to optimize CPP and to control ICH should be coordinated in the temporal progression of TBI. In addition to increasing the blood pressure pharmacologically to maintain adequate cerebral perfusion pressure, there is a need for strategies to reduce hemorrhage progression, and to address the harmful effects of the hemorrhage. To achieve this goal, an understanding of the pathophysiology of tICH is essential. Although there are significant differences between tICH and sICH, they share common processes and a review of the data in sICH could shed light on the mechanisms of injury in tICH.

This review will highlight some of the cellular pathways in ICH with an emphasis on the mechanisms of secondary injury due to heme toxicity and to events in the coagulation process, which are common to the different types of sICH and tICH.

Release of free heme

Heme is a major component of hemoproteins, including hemoglobin, myoglobin, cytochromes, guanylate cyclase, and nitric oxide synthase. Free heme is deposited in tissue only in pathological conditions. Hemorrhage, ischemia, edema, and mechanical injury damage are all processes that may result in the release of heme from hemoproteins [7]. Intracellular heme originates from cytoplasmic hemoproteins and from mitochondrial cytochromes located in neurons and glia [8]. Extracellular heme is released from dying cells and from extravasated hemoglobin from red blood cells [9]. The release of oxyhemoglobin (oxyHb) leads to superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) release as oxyhemoglobin undergoes auto-oxidation to methemoglobin. Free heme is degraded by heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2) into Fe^{2+} , CO, and one isomer of biliverdin, which rapidly reduces to free bilirubin. Free heme is lipophilic and enhances lipid peroxidation [10]. Free iron is also extremely toxic to cells (Huang et al, 2002; Kadoya et al, 1995; Panizzon et al, 1996). It reacts with H_2O_2 to form hydroxyl radicals, and degrades membrane lipid peroxides to yield alkoxy- and peroxy-radicals, which cause further chain reactions of free radical-induced damage [10, 11]. The result is oxidative damage to lipids, DNA, and proteins, leading to caspase activation and neuronal death [12]. Additionally, damage to endothelial cells causes BBB breakdown, resulting in vasogenic edema, increased ICP, and ischemia [13-15]. The effect of bilirubin formation after TBI is unclear. At low physiologic nanomolar concentrations in the healthy brain, bilirubin has potent anti-oxidative properties; but at high concentrations, it can act as a neurotoxin [7]. The level at which it is neuroprotective vs. neurotoxic is not clear, especially in the complex environment after TBI. The role of CO generation is controversial – it is beneficial by promoting relaxation of vascular smooth muscle and decreasing vasospasm [7].

Because of the potential harmful effects of hemoglobin (Hb) breakdown, HO-1 and HO-2 activity may be detrimental after TBI. HO inhibitors have been shown to reduce edema in models of ischemia, hemorrhage, and trauma [16-18]. However, there is also evidence that HO-1 is neuroprotective [16]. These discrepancies may result from the model used, and the brain region or even the cell type studied. In cell culture, for example, HO-1, induced in reactive astrocytes and microglia/macrophages, protects cortical astrocytes but not neurons from oxidative stress after exposure to Hb and H_2O_2 [17, 18]. Other investigations involve

the anti-oxidant activity of bilirubin/biliverdin redox cycling [19], and the administration of deferoxamine, a scavenger for ferric iron which improved spatial memory in a rodent model of ICH [20].

Haptoglobin (Hp), a glycoprotein which binds free Hb almost irreversibly, is a potential endogenous neuroprotectant after ICH [13, 21, 22]. In the Hb-Hp complex, the iron moiety is situated within the hydrophobic pocket of Hb, preventing its oxidative and cytotoxic activities [23]. Hp binding also facilitates the clearance of free Hb by monocytes and macrophages, and promotes iron recycling [24]. In addition to preventing Hb-induced oxidative cellular damage to cells, Hp-Hb binding protects Hb itself from oxidative damage. This is especially important since oxidative changes to Hb render it unable to bind to Hp, or to the Hb scavenger receptor CD163 on macrophages [25], which mediate Hb endocytosis and clearance. Interestingly, the presence of free Hb alone could induce a state of hypohaptoglobinemia [11], making the induction of Hp expression even more crucial. Hp level in the brain is low at baseline [11], but its expression can be induced in the brain and in peripheral blood by intracerebral injection of blood [11]. Over-expression of Hp, induced pharmacologically with sulforaphane (an activator of nuclear factor-erythroid 2-related factor (Nrf2), reduces brain injury after experimental ICH [11].

Oligodendrocytes appear to be the major producer of Hp in the CNS [11]. Since oligodendrocytes are closely associated with axons, they are uniquely situated to decrease the effects of heme toxicity on white matter tracts, which are situated along the path of blood extravasation during aSAH. In fact, Hp-overexpressing mice show better axonal integrity than Hp-null mice [11]. This function of oligodendrocytes adds to their known activities in protecting axons from excitotoxic and oxidative stress. In addition to protecting neurons, Hp also protects oligodendrocytes themselves from heme toxicity [11], ensuring continued intracranial production of Hp. Thus, Hp is a promising endogenous agent that may be utilized to limit Hb-mediated toxicity in ICH.

Activation of coagulation

Coagulation disorders are common after TBI, including both hypercoagulability and coagulopathy. In the presence of coagulopathy, there is a ten-fold increase in the risk of mortality from TBI, and a thirty-fold increase in significant morbidity. Tissue factor (TF), a protein present in endothelial cells and leukocytes and the primary physiological initiator of the coagulation cascade, is released into the general circulation following injury to brain vessels [26]. Cerebral vessels have a rich store of TF and play a central role in inducing CNS and systemic coagulopathy [26, 27]. The large amount of TF released can overwhelm normal control mechanisms that prevent excessive coagulation. With significant hemorrhage, anti-thrombin, clotting factors, and platelets are consumed. At the same time, the expression of plasminogen activator-inhibitor (PAI-1) expression is increased, inhibiting the fibrinolytic system [28]. Tissue hypoperfusion and activation of the protein C pathway have also been implicated in the early coagulopathy seen in TBI [29]. These factors contribute to DIC, seen in as much as 25% of patients with severe TBI. DIC-induced coagulopathy may develop within hours of injury [30]. On the other hand, TF-dependent activation of coagulation can cause both micro-vascular and macro-vascular fibrin thrombi formation. Excessive micro-thrombi formation may obstruct flow in small vessels, and is proposed to be a cause of delayed ischemia in investigations of aSAH [31]. Because of the complexity of the coagulation system with its myriad factors, thrombin will be highlighted here as an example of the multiple secondary effects of activation of the coagulation system.

Thrombin has been shown to be a potent inducer of microparticle release from platelets and endothelial cells [35]. MPs are small vesicles which consist of a small amount of cytosol and bilayer plasma membrane, and which express surface antigens from the cell of origin. MP

release occurs during many types of apoptogenic, procoagulant, or proinflammatory stimulation [32]. These stimuli trigger the migration of procoagulant phospholipids, such as phosphatidylserine, to the outer leaflet of the plasma membrane, with subsequent membrane budding which is released in the form of MPs. Phosphatidylserine creates an additional procoagulant surface for the assembly of clotting enzyme complexes on MP surfaces [28]. MPs also provide a reservoir of circulating TF, and enhance the catalytic efficiency of the TF/factor VIIa complex [28].

In addition to promoting fibrin clot formation, thrombin also mediates vasoconstriction through the thrombin receptor, PAR1, found on endothelial cells [33]. When cleaved by thrombin, activated PAR1 mediates vasoconstriction, and upregulates its own expression, increasing the vessel responsiveness to thrombin [33]. These processes potentiate vasoconstriction, which limits bleeding but can worsen ischemia after TBI. As is the case with many pathways reviewed, PAR1 activation also directly triggers inflammation [34] and increases BBB permeability, edema, and cell death [35].

Activation of Platelets

Beyond their crucial role in hemostasis, activated platelets have important secondary effects on the vasculature. The interaction of platelets with the exposed collagen of the blood vessel and with activated leukocytes contribute to platelet degranulation and to the formation of adhesion molecules in the endothelium [36]. These events trigger the release of eicosanoids and free radicals from granulocytes, increasing oxidative stress in the microenvironment of the hemorrhage. Platelets also directly contribute to vasoconstriction after ICH. Platelets and mast cells release 5-HT when activated. 5-HT has many actions, including increased vascular permeability [36], and vasoconstriction in large cerebral arteries but vasodilation in small cerebral arteries [37, 38]. It also stimulates the sensory fibers of the trigeminovascular system which release substance P (SP) antidromically [39], contributing to the effects of neurogenic inflammation. In experimental SAH, platelets also interact with perivascular nerves as follows: release of sensory fiber transmitters SP from these nerves stimulate the arachadonic acid cascade within platelets [40], contributing to inflammation through products of AA metabolism such as prostaglandins and leukotrienes.

Activation of Leukocytes

Leukocyte-endothelial cell interactions, mediated by intercellular adhesion molecule-1 (ICAM-1), lymphocyte function-associated antigen-1 (LFA-1), macrophage antigen-1 (Mac-1) and endothelial (E)-selectin, constitute another important element in the inflammatory response [41]. These interactions are believed to contribute to the development of vasospasm in aSAH. Infiltrating neutrophils may secrete TNF α and pro-inflammatory proteases, and generate ROS [42]. Dying leukocytes may stimulate macrophages to release pro-inflammatory mediators [43]. Mast cells migrate to vessel walls after SAH and release histamine when stimulated by bradykinin [44]. In studies of cerebral ischemia, leukocyte-endothelial cell adhesion helps to initiate and propagate reperfusion injury. Mice that have received neutralizing antibodies against leukocyte adhesion receptors and mutant mice that are genetically deficient in these adhesion receptors [45] experience less microvascular dysfunction and tissue injury following ischemia/reperfusion. If the same holds true for post-traumatic hemorrhage, then anti-leukocyte strategies should be explored as a therapeutic option.

Actions of Microglia, Astrocytes, and Oligodendrocytes

In studies of SAH, activated microglia have phagocytic activities which help to clear the hematoma. Thus, microglial activation and macrophage infiltration may be beneficial after hemorrhage. However, activated microglia also release cytokines [46-48], ROS [49, 50], and

nitric oxide [51, 52], all of which also contribute to hemorrhage-induced brain injury. Use of anti-microglial strategies, such as tetracycline derivatives, in animal models of ICH have resulted in reduced injury size, edema, and improved neurologic function [53], but have not been substantiated in clinical studies. Importantly, these therapies must maintain the beneficial actions of microglia while decreasing their pro-inflammatory activities.

Astrocytes modulate the neuronal response to brain injury through the production of angiogenic and neurotrophic factors. For instance, they influence neuronal sensitivity to glutamate toxicity by regulating the expression of the NMDA receptor subunit and the glutamate transporter excitatory amino acid carrier [54]. Interestingly, astrocytes could also modulate microglial ROS production [49] and thus play a role in limiting the harmful effects of microglial ROS release, without compromising the other positive effects of microglia activation. Oligodendrocytes also play an important role. As discussed earlier, one of their responses to hemorrhage is the increased expression of haptoglobin [11], which binds free heme and limits heme-induced toxicity.

The vascular response

The different cells within the blood vessels respond differently to the presence of extravascular blood [55]. Cerebral arteries contain three structural layers: the external layer (the adventitia) contains axons of the perivascular nerves in a collagen sheath; the media contains smooth muscle; the internal layer (the intima) contains endothelial cells and the basement membrane. Oxyhemoglobin, released by lysed red blood cells, is pathogenic for all three layers [55]. In the adventitia, the loss of nerve fibers has been reported after sICH [56, 57]. This type of denervation could be expected to result in loss of neurogenic control of cerebral arteries [55] and impairment of autoregulation. In the media, myonecrosis occurs with loss of contractile protein in the smooth muscle [58, 59]; additionally, the amount of interstitial collagen increases [58]. Together these processes contribute to arterial narrowing which is not vasospasm-mediated [60]. In the intima layer, matrix metalloproteinases (MMPs) are released after hemorrhage. MMPs degrade the basement membrane and tight junction proteins, resulting in disruption of the BBB. Local substances released by multiple cell types also affect the permeability of the BBB [61]. Perivascular nerve fibers release CGRP, 5-HT, and SP which contribute to mast cell release of histamine [55], which increases inflammation.

Endothelial cells themselves release substances that may be detrimental in the setting of TBI. An example is endothelin, a potent vasoconstrictor, which helps to limit hemorrhage but may aggravate ischemic secondary injury after TBI. After ICH, endothelial cells also up-regulate the expression of receptors to SUR1 and to angiotensin I (AT1) [62]. These responses also contribute to vasoconstriction. SUR1 [63] and ET-1 [55] have the additional effect of increasing BBB permeability. Specific antagonists to SUR1, AT1 receptors, and ET-1 [55, 62, 63] have been shown to reduce cerebral edema and improve outcome in animal models of ICH. These are only a few examples of the diverse responses triggered in cerebral endothelial cells after ICH. These responses have been studied most extensively in the context of vasospasm after aSAH. It has been pointed out that vasospasm also plays a role after the different types of hemorrhage after TBI [64, 65], but it is unclear whether the treatment of post-traumatic vasospasm improves outcome.

Recent studies show that endothelial microparticles (MP) are released into the circulation at high proportions in TBI. In TBI, the proportion of endothelial MPs (compared to platelet MPs) was found to be higher than in many other disease entities, including spontaneous SAH, which is known to cause extensive cerebral endothelial damage [28]. This finding suggests that severe TBI and tICH result in more extensive endothelial activation than sICH, exacerbating the prothrombotic and proinflammatory signaling pathways in TBI.

Conclusion

Intracerebral hemorrhage initiates many cellular responses beyond those that restore hemostasis, and these responses may contribute to secondary brain injury (see Figure). ICH is often accompanied by increased ICP, ischemia, oxidative damage, vasogenic edema, and cytotoxic edema. These processes disrupt mitochondrial energetics, and lead to neuronal cell death. Heme-toxicity, iron-toxicity, and activation of coagulation are obviously key elements in both sICH and tICH. Thus, the data on cellular mechanisms in sICH may be applicable to the understanding of tICH. However, there are also significant differences among the different types of traumatic and spontaneous ICHs. These differences include: the distribution of the various hemorrhage subtypes (subdural, subarachnoid, intraparenchymal, or intraventricular) in spontaneous vs. traumatic bleeds, the extent and rate of hemorrhage progression, the degree and duration of increased intracranial pressure, the presence of blood beneath the subarachnoid layer vs. the dura. Additionally, the presence of concomitant injuries in TBI may influence the response to ICH. Thus, specific research of the pathophysiology of tICH and the recovery process is needed in order to identify specific therapeutic targets for these processes.

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Figure 1.
Representative pathways of secondary brain injury after ICH.