Challenges for intraventricular hemorrhage research and emerging therapeutic targets

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To cite this article: Thomas Garton, Ya Hua, Jianming Xiang, Guohua Xi & Richard F. Keep (2017) Challenges for intraventricular hemorrhage research and emerging therapeutic targets, Expert Opinion on Therapeutic Targets, 21:12, 1111-1122, DOI: 10.1080/14728222.2017.1397628

To link to this article: https://doi.org/10.1080/14728222.2017.1397628
Challenges for intraventricular hemorrhage research and emerging therapeutic targets

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ABSTRACT

Introduction: Intraventricular hemorrhage (IVH) affects both premature infants and adults. In both demographics, it has high mortality and morbidity. There is no FDA approved therapy that improves neurological outcome in either population highlighting the need for additional focus on therapeutic targets and treatments emerging from preclinical studies.

Areas covered: IVH induces both initial injury linked to the physical effects of the blood (mass effect) and secondary injury linked to the brain response to the hemorrhage. Preclinical studies have identified multiple secondary injury mechanisms following IVH, and particularly the role of blood components (e.g. hemoglobin, iron, thrombin). This review, with an emphasis on pre-clinical IVH research, highlights therapeutic targets and treatments that may be of use in prevention, acute care, or repair of damage.

Expert opinion: An IVH is a potentially devastating event. Progress has been made in elucidating injury mechanisms, but this has still to translate to the clinic. Some pathways involved in injury also have beneficial effects (coagulation cascade/inflammation). A greater understanding of the downstream pathways involved in those pathways may allow therapeutic development. Iron chelation (deferoxamine) is in clinical trial for intracerebral hemorrhage and preclinical data suggest it may be a potential treatment for IVH.

1. Introduction

Intraventricular hemorrhage (IVH) occurs when blood from a cerebral hemorrhage expands into the brain ventricular system. This can happen via a variety of mechanisms with both the elderly and preterm neonates being high-risk demographics. In adults, IVH occurs as an extension of intracerebral hemorrhage (ICH) in ~50% patients and it is an independent predictor of worse outcome [1]. In preterm neonates, IVH typically occurs as a result of germinal matrix hemorrhage (GMH), a brain region abutting the lateral ventricles. This combined phenomenon is referred to as GMH-IVH and affects over a third of extreme preterm birth infants with mortality rates of over 50% in that population [2]. Those that survive often develop significant neurological sequelae such as cerebral palsy, developmental delay, deafness, and blindness. In particular, post-hemorrhagic hydrocephalus (PHH) is a common comorbidity with IVH in neonatal populations and an independent predictor of poor prognosis [3].

Currently, no treatment has demonstrated improved clinical outcome in either adult or neonatal IVH. Current treatments focus on methods of CSF drainage to limit PHH [4,5]. While there is continued interest in the use of intraventricular thrombolytics to remove IVH, the recent large clot lysis: evaluation of accelerated resolution of intraventricular hemorrhage III (CLEAR III) trial in adult IVH, failed to show a significant benefit on neurological outcome with tissue plasminogen activator (tPA), although mortality was reduced [6]. As yet, no neuroprotectant or any approach to improve brain recovery after IVH has shown clinical benefit.

Preclinical studies on IVH have, however, identified several potential therapeutic targets for reducing brain injury. This review examines the current state of research on IVH in both adults and neonates. Exploration of those findings may give important information for improving our ability to care for those with IVH.

2. IVH modeling

2.1. Adult IVH models

A variety of models have been utilized to investigate adult IVH. Pang et al. injected pre-clotted autologous blood directly into the lateral ventricles of dogs [7]. This model demonstrated ventricular enlargement simulating PHH. Another porcine model was created to study post-hemorrhagic ventriculomegaly [8]. This model also employed direct injection of autologous blood into the ventricles, but co-injected thrombin to accelerate coagulation. Despite the relative successes of these two models at developing enlarged ventricles, the majority of current IVH animal models use small rodents such as rats or mice.

In rodents, the primary method of simulating hemorrhage is via autologous blood injection. Some models utilize direct intracerebroventricular (ICV) injection [9] while others attempt to simulate combined ICH and IVH by injecting into periventricular brain regions [10]. Ventricular injection provides a greater degree of control over the analysis by solely investigating the effects of intraventricular blood, but a parenchymal...
Improving brain recovery after IVH is also a therapeutic target, e.g. there are a number of potential strategies to try and limit the occurrence and size of IVH including targeting cerebrovascular remodeling and stability.

Intraventricular hemorrhage results in a physical disruption that is a target of hematoma evacuation and efforts to accelerate hematoma resolution pharmacologically. Intraventricular hemorrhage also causes secondary injury via the release of factors from the hematoma that induce damage, neuroinflammation and cause CSF hypersecretion. Such factors include hemoglobin, iron and thrombin. These factors (or their downstream mediators) may be therapeutic targets for reducing IVH-induced brain injury.

Improving brain recovery after IVH is also a therapeutic target, e.g. via stem cells.

This box summarizes key points contained in the article.

2.2. Neonatal IVH models

As in adults, direct ICV injection of blood and blood components such as hemoglobin and iron into neonatal rodents has been used to model IVH [14,20]. These models have allowed investigation of the role of blood components in the formation of PHH and in neuronal degeneration. Neonatal GMH-IVH has also been studied using periventricular injections of collagenase in postnatal day-six rats [21]. Ventricular enlargement following blood extension into the ventricles was observed. Similarly, another study utilized collagenase injection directly into the germinal matrix [22]. That study did not investigate for morphological changes, but rather studied cognitive and sensorimotor function.

For neonatal IVH, there are also models of genetic and stress-induced IVH. One spontaneous GMH-IVH model is a transgenic mouse embryo model wherein vascular endothelial growth factor (VEGF) overexpression is induced specifically in the germinal matrix via the tetracycline regulatory system [23]. VEGF is involved in angiogenesis and its overexpression leads to an outgrowth of weak vasculature that is prone to rupture. This model reported 90% incidence of intracranial hemorrhage that extended into the ventricles, but also had low efficiency with 80% of embryos dying before birth. Despite this, such a model is encouraging and further efforts should be made into creating models of spontaneous IVH.

There are other genetic mutations that cause vascular disruption and neonatal or fetal ICH. Gould et al. reported a semi-dominant mutation in procollagen type IV alpha 1 that causes ICH and death within one day of birth [24]. Similarly, McCarty et al. found that mice null for αv integrin develop ICH in utero and die soon after birth [25]. Whether it is possible to induce IVH by manipulating such genes specifically in the periventricular zone merits investigation.

There are also models of stress-induced GMH-IVH. Ballabh and colleagues have developed a model where rabbit pups are delivered prematurely and then treated with glycerol to induce hyper-osmolality [26]. The majority of pups (~80%) develop IVH, and they also have periventricular cell death, axonal damage, neuroinflammation and behavioral deficits, and approximately half the animals develop PHH [26]. In newborn beagle puppies, GMH-IVH can be induced by a number of stressors, hypercarbia, hypertension, and hypotension with volume re-expansion [27,28]. These GMH-IVH models have important clinical relevance, but there can be difficulties determining whether the cause of particular injuries is the GMH or the IVH (or the combination), which has therapeutic consequences.

2.3. In vitro models

There are no in vitro models that fully replicate an IVH. However, standard cell culture techniques have been used to examine the impact of clot-derived factors (e.g. hemoglobin, iron, thrombin) on multiple types of brain cells (e.g. neurons, astrocytes, microglia, endothelial cells). Developments in inducible pluripotent stem cells (iPSCs) will now allow such experiments in patient-derived cells [29]. Similarly, the use of those cells to produce brain organoids may allow dissection of the effects of clot-derived factors on cell–cell interactions. Such organoids produce ventricle-like structures [30].

3. Prevention

There are multiple potential strategies to reduce IVH occurrence or size. The first is to reduce the risk factors for IVH. In adults, the major risk factor for ICH (the underlying cause of IVH) is hypertension and there is evidence that increased access to antihypertensive medication may decrease ICH incidence [1]. As occurs in other vascular beds, hypertension causes cerebrovascular remodeling and altered function. This includes misaligned smooth muscle cells, reduced autoregulatory ability and blood–brain barrier leakage that may
precede hemorrhage [31]. Targeting those changes [31], as well as lowering blood pressure, may be a way of reducing adult ICH/IVH. A second, and growing, cause of ICH is anticoagulant use. Warfarin use is associated with IVH risk, hematoma volume, and poor prognosis in adults suffering from ICH [32]. However, for anticoagulant use, there is a trade-off between increased hemorrhagic stroke risk and reduced ischemic stroke risk. Reduced platelet activity is also associated with more IVH in adult ICH patients [33]. A third major cause of ICH is cerebral amyloid angiopathy, but because of the generally lobar location, it is not a major cause of IVH [19].

In neonates, the major risk factor for GMH-IVH is prematurity as the germinal matrix almost completely involutes at about gestational week 33 in humans. Preventing premature birth is a complicated area of research that has been reviewed elsewhere [34], but the number of premature infants, and especially extremely premature infants, that are surviving after birth is increasing [35]. Cerebral blood flow fluctuations in neonates can add stress to the germinal matrix vasculature and are seen as a potential factor underlying neonatal IVH. Elimination of such fluctuation via intravenous pancuronium infusion has long been shown to reduce IVH incidence [36]. Current use of synchronized ventilator modes in neonatal care also reduces the amount of cerebral blood flow fluctuation [37].

Another potential strategy to reduce IVH occurrence is to strengthen potentially fragile vessels [38]. Prenatal administration of glucocorticoids reduces the severity and frequency of IVH, as these can stabilize the germinal matrix vasculature [39]. However, glucocorticoids can have side effects by affecting brain development [40]. There is a growing understanding of the developmental regulation of brain angiogenesis and barrierogenesis [41]. There has been a dissection of the roles of pericytes and astrocytes, and signaling pathways including platelet derived growth factor B, sonic hedgehog and angioptin in strengthening the links between brain endothelial cells [41]. Further studies are needed to determine whether modulating such pathways might be a way of reducing IVH without affecting brain development.

A third potential approach is to limit the amount of bleeding from already ruptured vessels. In adult ICH, ~40% of patients undergo hematoma expansion within the first 24 h [42] and there has been considerable effort to try and reduce that expansion by using hemostatic agents or by acutely reducing blood pressure. None of these trials have shown a significant improvement in neurological outcome although there is evidence of reduced hematoma expansion. The Factor VIIa in Acute Intracerebral Hemorrhage (FAST) trial showed reduced hematoma expansion over the first 24 h [43] and a secondary analysis of the Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trials (INTERACT 1 and 2) found that blood pressure reduction reduced ICH plus IVH hematoma expansion [44].

A fourth potential strategy is to identify whether there are specific genetic vulnerabilities for IVH that might be amenable to therapeutic intervention. The majority of such efforts have focused on coagulation or fibrinolytic genes, with special focus on the Factor V Leiden (Arg506Gln) and prothrombin (G20210A) mutations [45]. These mutations have been associated with increased risk of GMH-IVH in preterm birth infants [46–48]. Additionally, certain haplotypes of the gene encoding VEGF A are correlated to increased incidence of IVH in preterm infants [49]. However, as of yet, few if any preclinical studies have been reported that manipulate these genes, so no causal relationship has been demonstrated.

For adult IVH, in particular, preclinical work on prevention with any of these strategies is hampered by the relative paucity of spontaneous ICH models. There has been work on examining the impact of different anticoagulants on ICH after collagenase injection [50]. Experiments examining the impact of such anticoagulants on combined ICH/IVH models are warranted although there are concerns that the collagenase models may differ from human hemorrhage (bacterial protein and underlying mechanism of vessel disruption [51]).

4. IVH-induced brain injury

After ICH/IVH, there is both a primary injury, caused by the physical presence of the blood within the brain, and secondary injury caused by the effects of neurotoxic compounds released from the hematoma and the brain response to blood (e.g. inflammation). The time windows for these injury processes differ and most preclinical research has focused in secondary injury.

4.1. Physical effect of IVH

Three physical effects of an intraventricular hematoma are the displacement of neural tissue (mass effect), increased intracranial pressure (ICP) and blocking of the CSF flow pathway. The immediate physical impact of an IVH will involve stretching of the wall of the ventricles and periventricular structures. The importance of such stretch in IVH-induced brain injury has not been well studied preclinically. It is known that there can be extensive loss of the ependymal cells that line the ventricle walls after ICH [52], but the relative importance of physical stretch versus clot-derived factors is still unclear. Thus, for example, it is known that ICV injection of iron can cause ependymal damage [12] without having a mass effect.

In adults, the volume of the intracranial space is fixed, so the presence of blood increases ICP unless there is a compensatory fluid movement (e.g. CSF displacement). The CLEAR III trial reported ICPs of >30 mmHg in 30% of patients with ICH/IVH who didn’t require a CSF shunt and ~44% of patients who did [53]. High ICP may impact cerebral blood flow and cause brain herniation. In neonates, there is some skull flexibility that may limit the increases in ICP caused by increased intracranial volume. The role of transient global ischemia on brain injury after IVH has received little attention in preclinical models.

Another effect of an IVH is that it can block the CSF drainage pathway, at least transiently [9,54], either within the ventricular system or at the CSF outflow sites. This may lead to, or contribute to, PHH. It should be noted that as well as a physical block by the blood clots, there may be other alterations to the CSF pathways contributing to PHH (e.g. outflow site fibrosis). Very recent evidence also indicates that CSF secretion rate is increased after experimental IVH via toll-like receptor (TLR)-4 activation. That causes activation of Ste20-type stress kinase (SPAK) which
phosphorylates and activates Na/K/Cl cotransporter (NKCC)-1 at the apical surface of the choroid plexus epithelium [55]. CSF hypersecretion may help clear potentially harmful clot-derived factors from the brain, but it may also contribute to PHH particularly if CSF absorption is impaired. Thus, targeting the TLR4, SPAK, NKCC1 pathway may be a method of reduced PHH [55].

4.2. Mass effect

Reducing the mass effect after IVH requires clearing the hematoma from the ventricles. Identifying methods of clearing IVH has been a goal in many preclinical and clinical studies [5,6,56]. This has generally been performed by ICV injection of a thrombolytic such as tPA or urokinase to lyse the hematoma. Whether fibrinolytics improve outcome is still uncertain. A meta-analysis of intraventricular fibrinolytic therapy in adults found that tPA administration reduced ventricular dilation and mortality, and improved functional outcomes in adults [57]. One recent meta-analysis observed that using fibrinolytics in conjunction with external ventricular drainage provided the best improvement in mortality rates [58]. However, another meta-analysis reported that while fibrinolytic treatment did reduce mortality, the data failed to reach significance [56]. The very recent CLEAR III trial compared outcomes ICV tPA versus placebo in patients with an EVD. It found that tPA significantly reduced IVH volume and mortality but it did not improve the number of patients with good functional outcome (as assessed by modified Rankin score, the primary end point of the trial 6).

In premature infants, there have been multiple studies of ICV fibrinolytics (tPA, urokinase, streptokinase) to reduce the need of shunt placement after PHH [5]. While there have been some studies that have suggested a benefit, others have not and have shown increased secondary IVH. Thus, currently, the clinical recommendation is not to use fibrinolytics in children with PHH [5].

In relation to IVH clearance studies, it is important to note two points. First, that this approach may have effects on both primary (mass effect) and secondary injury (removing the clot a source of neurotoxic factors). Second, there is the question of timing. Giving a thrombolytic in a patient with an intracranial bleed might result in new bleeding. Thus, the CLEAR III trial delayed tPA administration until the clot was stable on CT scan to limit this possibility. This meant that the average time from ictus to randomization between tPA and saline groups was 52 h [6] which may reduce the effect on the initial primary injury.

There have been few preclinical studies examining the effects of fibrinolytics on IVH [8,59]. Such studies may be useful for direct comparison of tPA and urokinase. It has been proposed that urokinase lacks some of the proinflammatory properties of tPA making it a potentially preferable choice [56]. In addition, preclinical models may be used to study combination therapies (e.g. a fibrinolytic and a neuroprotectant).

4.3. Imbalance in CSF secretion and absorption

The choroid plexuses are responsible for the majority of CSF secretion [60,61] and reducing that secretion is a target for IVH therapy and particularly for ameliorating PHH. The choroid plexus is a secretory epithelium where the epithelial cells are linked by tight junctions and contain a wide array of ion transporters as well as water channels (primarily aquaporin 1, AQP1). The epithelium is involved in vectorial ion transport that can drive water transport [60]. Clinically, a combination of acetazolamide (a carbonic anhydrase inhibitor) and furosemide (a Na/Cl cotransport inhibitor) has been evaluated as a method of reducing CSF secretion but it does not reduce the need for shunt placement in preterm infants with PHH and actually increased neurological morbidity [5]. Currently, therefore, CSF is regulated in PHH patients via one of several CSF drainage methods [5].

Our greater understanding of ion and water transport across the choroid plexus is, however, suggesting some new therapeutic targets including the tight junction protein claudin-2 and the water channel AQP1. Unlike the claudin-5 present in the tight junctions of the blood–brain barrier, the claudin-2 present in choroid plexus epithelial cells is ion and water permeable [60] and may represent a paracellular route for fluid movement. AQP1 is important in choroid plexus fluid secretion [62] and aquaporin inhibitors are beginning to be developed [63].

It should be noted that surprisingly little is known about how the choroid plexuses are impacted by IVH [64]. Such changes may impact fluid secretion (e.g. aquaporin expression [65]) and after therapeutic targets.

4.4. Blood components and brain injury

A significant volume of recent research on IVH has indicated that secondary damage caused by blood components is responsible for much of the observed injury. After a hemorrhage, erythrocytes can lyse and release their potentially toxic contents into the ventricular system. Moreover, other blood components such as elements of the coagulation cascade and immune cells can independently induce brain damage. There are likely similarities in injury mechanisms between adult and neonatal IVH. However, it should be noted that for ischemic stroke, there are age-dependent differences in the impact of microglia and neuroinflammation [66]. Given the amount of preclinical research that has aimed at elucidating blood-component-based mechanisms of injury, several therapeutic targets have been identified. The following section will describe the mechanisms of blood-derived toxicity.

4.4.1. Erythrocyte lysis, hemoglobin, and its degradation products

Early after IVH, erythrocytes can start to lyse. ICV injection of lysed erythrocytes causes brain injury indicating that there are neurotoxic compound(s) within the red blood cell [12]. This also suggests that erythrocyte lysis may be a therapeutic target in IVH. The mechanism of such lysis after IVH has not been well established. It may reflect gradual energy depletion in the erythrocyte, but it may also result from complement activation and insertion of membrane attack complex in the cell membrane. The extent to which erythrocytes lyse before they can undergo phagocytosis by microglia/macrophages may have an important impact on brain injury.

One erythrocyte component released into the ventricular system is hemoglobin. Free hemoglobin in extracellular spaces has been shown to exhibit cytotoxic effects and increase the
inflammatory response \[67\]; consequently, the role of hemoglobin in post-hemorrhagic damage has been extensively investigated (Figure 1). ICV injection of hemoglobin results in inflammatory responses characterized by increased levels of the proinflammatory cytokine tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) in the CSF, as well as by periventricular brain damage \[68,69\]. The ependymal cells that line the cerebral ventricles are injured by IVH and iron resulting in areas of denudation \[12,52\]. This may facilitate the movement of hemoglobin deeper into the periventricular brain. The periventricular region includes the sub-ventricular zone, a site of neurogenesis in neonates and mature adults. The presence of ventricular hemoglobin can also affect more distal brain region. There is significant c-Jun N-terminal kinase (JNK)-linked neurodegeneration in the hippocampus within three days of an ICV injection of hemoglobin \[20\]. The mechanism of distal damage has not been fully elucidated. Possibilities include diffusion of hemoglobin to the hippocampus, inflammatory injury, or damage of nearby axons that belong to distant soma.

Hemoglobin is capable of inducing PHH as well as cellular degeneration. In neonatal rat models of IVH, ICV injections of hemoglobin result in significant ventriculomegaly compared to injections with artificial CSF \[14\]. Again, the mechanism of this injury is not fully known. It is possible, however, that the pathway includes the choroid plexus, which is not only the primary site of CSF production, but which is also a crucial component of the blood-CSF barrier \[68\]. Karimy et al. found the IVH induced CSF hypersecretion via activation of TLR4 on the choroid plexus epithelium and stimulation of NKCC1 activity \[55\]. Methemoglobin and heme, a hemoglobin degradation product, can activate TLR4 \[70\]. In addition, ICV injection of hemoglobin can

Figure 1. Schematic showing effects of IVH related to erythrocyte lysis and hemoglobin release within the ventricular system. IVH causes ependymal damage and this may facilitate the penetration of hemoglobin from CSF into brain parenchyma. Hemoglobin may then be taken up into microglia/macrophages via the CD163 receptor, particularly when hemoglobin is complexed to haptoglobin. Alternately, heme released from hemoglobin may be taken up by microglial cells via the CD91 receptor. Inside microglia, hemoglobin/heme is degraded by heme oxygenase-1 (HO-1) to iron, carbon monoxide and biliverdin. HO-1 is inducible and it is markedly upregulated after cerebral hemorrhage. In microglia, iron produced by HO-1 can be chelated by ferritin reducing iron-mediated damage. Recent evidence indicates that neurons can also express CD163 and hemoglobin uptake into those cells may be more toxic due to a lack of ferritin. Neurons express a constitutive form of heme oxygenase, HO-2. Hemoglobin in CSF can also cause damage to the choroid plexus and this may impact CSF homeostasis, result in infiltration of leukocytes and potentially contribute to hydrocephalus, an understudied area.
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dimers which may be cell types in the brain, this iron is quickly sequestered by biliverdin, carbon monoxide, and ferrous iron [72]. AQP alterations have been linked to the induction of hydrocephalus [72]. In a preterm rabbit pup model, IVH resulted in choroid plexus AQPs1 and AQPs5 protein levels [65]. In certain studies, it has been shown that AQPs5 overexpression can result in increased RAS-related cell proliferation [73]. If such cell proliferation should occur in the choroid plexus, it may result in increased CSF production and consequential buildup of ICP. Thus, there is a need for greater understanding of the role of choroid plexus AQPs in PHH.

The mechanism underlying hemoglobin-induced damage is likely tied to its degradation. Within the ventricular system, hemoglobin naturally dissociates into αβ dimers which may be bound by haptoglobin molecules derived from the hematoma. Haptoglobin is a plasma protein with very affinity for hemoglobin [74] that plays a key role in clearing intravascular hemoglobin. While the brain may start to produce haptoglobin after a cerebral hemorrhage [75], normal adult CSF levels of haptoglobin are very low, meaning that there may be insufficient haptoglobin, at least initially, to fully scaveng free hemoglobin after hemolysis [76]. Moreover, plasma haptoglobin levels in neonates are also extremely low; this results in an inability to quickly bind free hemoglobin, resulting in the higher morbidity rates of neonatal hemorrhage [77].

Once securely bound, the haptoglobin-hemoglobin complexes undergo receptor-mediated endocytosis via CD163, a protein expressed in microglia and macrophages, and recently identified in neurons [20, 78]. After endocytosis, haptoglobin and hemoglobin dissociate, and heme is released, which is subsequently degraded by the heme oxygenase (HO) to form biliverdin, carbon monoxide, and ferrous iron [79]. In certain cell types in the brain, this iron is quickly sequestered by ferritin. However, in cell types lacking ferritin, such as neurons, this Fe2+ can participate in oxidative Fenton reactions. In that reaction, ferrous iron reacts with hydrogen peroxide to produce dangerous radical oxygen species:

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^-
\]

The resulting ferric iron is free to be reduced back to Fe2+ by cellular reducing agents. Once in the ferrous form, the reaction can begin again with a new equivalent of hydrogen peroxide, thus continuously generating reactive oxygen species.

Due to its role in the endocytosis of hemoglobin into cells like neurons that lack the appropriate iron sequestration systems, CD163 has been suggested to be a target for reducing neuronal injury after IVH [80]. However, microglial and macrophage CD163 has been shown to be beneficial for clearing hemoglobin from the extracellular space and thereby decreasing injury after cerebral hemorrhage [81]. This creates a therapeutic dilemma: any treatment that attempts to address neuronal expression of CD163 must be careful to avoid decreasing CD163 levels on microglia/macrophages. The ability of iron from hemoglobin to participate in radical reactions has made iron a focus of preclinical studies investigating hemoglobin during IVH. ICV injection of lysed red blood cells induces both HO-1 expression in brain, an enzyme that degrades heme and releases iron, and ferritin, the primary iron sequestration protein in brain [12]. Convincing evidence of the role of intra-hemoglobin iron came from Strahle et al. who demonstrated that injection of protoporphyrin IX (essentially an iron-less heme compound) does not induce hydrocephalus while iron and hemoglobin both did [14], implicating iron as the key component of the heme moiety in the mechanism behind PHH. Moreover, it is possible that this iron may be released into the CSF via iron export proteins such as ferroportin. In a clinical study of infants with PHH, 75% had free iron present in their CSF, while no control subjects had such iron [82]. Significant evidence supports the notion that the accumulation of iron following IVH results in ventricular dilation and brain damage [10]. Moreover, it has been shown to correlate with brain edema, neuronal cell death in the basal ganglia, and motor function deficits [83]. It has also been suggested that iron induces hydrocephalus by activating the Wnt signaling pathway after IVH, which is closely implicated in subarachnoid fibrosis in chronic hydrocephalus [84]. This finding warrants further investigation.

Deferoxamine (DFX) is a ferric ion chelator that is used clinically for systemic iron overload and that shows promise as a treatment for cerebral hemorrhage [85, 86]. In intracranial hemorrhage, DFX has beneficial effects on iron-induced edema [87], neuronal death [88], hippocampal degeneration [20], and inflammation [87, 89, 90]. A phase II clinical trial is ongoing for ICH (Intracerebral Hemorrhage Deroxamine Trial – iDEF Trial; NCT02175225). In models specific to IVH, DFX has been shown to reduce the amount of cell death and neuronal degeneration in peri-ventricular areas [10, 11, 20].

One effect of DFX is to ameliorate PHH [10, 91]. DFX co-injection reduces ventricular enlargement following IVC injection of lysed erythrocytes [12], hemoglobin, and iron injection in both adults and neonates [14]. While it is most likely DFX acts via iron chelation, it is possible that it can also affect important signaling pathways [92]. The Wnt signaling pathway plays a role in the coagulation cascade, and may be involved in the formation of obstructive non-communicating hydrocephalus following IVH. DFX has been shown to counteract Wnt activity following IVH [84].

Minocycline is another commonly studied preclinical treatment for intracranial hemorrhage due to its ability to chelate iron and inhibit microglia. Like DFX, minocycline has been repeatedly demonstrated to diminish damage in a variety of intracranial hemorrhage models [93, 94]. In cell cultures, it has been shown to reduce iron-induced cortical neuron degeneration with greater efficiency than DFX [95]. Guo et al. that demonstrated the efficacy of minocycline at diminishing iron accumulation in an experimental GMH-IVH model, resulting in decreased brain edema, hydrocephalus, and brain damage [83]. The mechanism of its action was likely iron based in nature, as it additionally reduced ferritin upregulation following the hemorrhage, but they may also be beneficial effects on microglia activation. Minocycline should be more rigorously evaluated as a potential treatment for iron-induced injury following IVH.
4.4.2. Coagulation components

Plasma, as well as erythrocyte, components play a role in IVH-induced brain injury. Significant among these are the elements of the coagulation cascade including prothrombin/thrombin (Factor IIa). Thrombin is produced following the cleavage of prothrombin, a process upregulated during hemorrhage. Thrombin functions primarily by cleaving fibrinogen into insoluble fibrin to prevent bleeding. However, following ICH and IVH, other thrombin functions can lead to detrimental outcomes [96] (Figure 2). For example, recently, Klebe et al. [97] found that a thrombin antagonist, dabigatran, reduced hydrocephalus and behavioral deficits in a rat GMH model.

Some of the functions of thrombin involve protease-activated receptors (PARs). PAR-1, a G-protein-coupled receptor, is directly activated by the serine protease activity of thrombin. Following its activation, PAR-1 can trigger multiple biological cascades [96]. Increased thrombin production during IVH results in PAR-1 activation which can damage the ependymal wall and lead to the induction of hydrocephalus [13]. The Src family of kinases are activated by the thrombin-PAR-1 system and are responsible for the phosphorylation (activation) of metalloproteinases, such as matrix metalloproteinase-9, which are known to be a significant cause of damage in other types of intracranial hemorrhage, although their specific roles in IVH and clot clearance are yet to be investigated [98].

Some treatments identified by studies investigating thrombin and PAR-1 following IVH include SCH79797, a PAR-1 antagonist [99]. This antagonist has been shown to significantly reduce ependymal wall damage following experimental IVH [13]. Inhibition of Src family kinases (and potential subsequent metalloproteinase activation) with PP2 blocks brain edema and blood brain barrier disruption [98]. In subarachnoid hemorrhage animal models, thrombin-induced inflammation has been linked to transforming growth factor β (TGF β) [100]. Normally associated with platelets in the blood, TGF β can be released into CSF following platelet extravasation during IVH; it has suggested that TGF β could play some role in the creation of obstructive hydrocephalus [101]. Recent studies of kaolin-induced hydrocephalus indicate that decorin, an antagonist of TGF β, can reverse ventriculomegaly and white matter injury [102,103]. However, further research into this topic will be necessary in order to fully elucidate the mechanism by which TGF β is involved in hydrocephalus following IVH.

Another part of the coagulation cascade that may play a part in IVH-induced brain injury is fibrinogen (Factor I). While the conversion of fibrinogen to fibrin by thrombin is essential for hemostasis, those clots can obstruct the passage of CSF. In addition, even un-cleaved fibrinogen may have negative effects once released into the ventricles. Evidence indicates that extravascular fibrinogen can induce powerful inflammatory response, activating microglial via the CD11b/CD18 receptor [104], and it may also play a role in subsequent brain injury.

Therapeutically, the targeting elements of the coagulation cascade in IVH poses significant problems. While they may contribute to brain injury, they also play a vital role in hemostasis. It may be possible to ameliorate this difficulty by targeting downstream mediators, e.g. targeting thrombin-mediated PAR-1 receptor activation while not affecting fibrinogen cleavage, or targeting fibrinogen-mediated microglial activation.

4.4.3. Other blood components

Most attention has focused on the role of hemoglobin/iron, prothrombin/thrombin, and fibrinogen/fibrin as clot-derived factors that may cause brain injury. However, it should be noted that other erythrocyte, plasma, and platelet-derived factors may impact surrounding brain tissue and this merits further investigation. Thus, for example, carbonic anhydrase from erythrocytes...
has been implicated in inducing brain injury after ICH [105]. Similarly, recent evidence has shown that lysophosphatidic acid, present in serum, can induce hydrocephalus and impair ependymal integrity [106]. Lysophosphatidic acid is produced by activated platelets [107] and the role of platelets in IVH-induced brain injury has received very little attention.

4.5. Neuroinflammation

Many of the clot-derived factors described above have proinflammatory properties (e.g. thrombin, fibrinogen, hemoglobin). After IVH, there is an upregulation of proinflammatory cytokines, microglial activation, and leukocyte infiltration into brain [108,109]. Microglia are the resident brain immune cells that upon activation can have a range of phenotypes. Broadly, these include polarization into an ‘M1’ phenotype that is generally proinflammatory, or ‘M2’ phenotype that is associated with the resolution of inflammation [110]. Following IVH, microglia with both M1 and M2 polarization accumulate in the periventricular regions beginning ~48 h post-hemorrhage [109]. Excessive microglial activation after injury to the immature brain has been linked to impairment and even development of cerebral palsy [111,112]. The inflammatory response after IVH also involves the infiltration of circulating leukocytes into brain [109]. Following IVH, very-low-birth-weight infants exhibit increased total leukocyte counts [113]. Inhibiting microglia activation/polarization and blocking leukocyte infiltration into brain are potential therapeutic targets in IVH. It should be noted, however, that neuroinflammation has an important role in brain repair as well as inducing brain injury and that the effects of inflammatory cells in brain injury may vary with age [66].

5. Preclinical findings on damage repair

While the majority of preclinical studies have focused on blocking or reducing IVH-induced brain damage, some studies have investigated the possibility of enhancing brain repair. These include examining enhancing neurogenesis, stem cell therapy, and reversal of hyaluronan buildup.

In neonates, IVH is generally caused by hemorrhage in the germinal matrix, a region that is a source of new neurons and glial cells in the developing brain. Attempts to repair this region, and thereby reduce developmental impairment, have included using recombinant erythropoietin (rEPO), which not only stimulates red blood cell production but also displays neuroprotective capabilities [114]. rEPO administration can enhance neuro- and oligodendrogenesis in neonates with white matter injury [115]. The efficacy of rEPO at improving the long-term cognitive outcomes of infants suffering from IVH is currently under study [116]. Unfortunately, an earlier large prospective clinical trial studying rEPO treatment in pre-term birth infants demonstrated that while rEPO is safe to use, it does not decrease IVH incidence or mortality rates [117]. Nevertheless, further investigation into rEPO as a treatment for IVH-induced damage is warranted.

An alternative approach is to use stem cell therapy. Currently, the main goal of such therapy is to modulate the immune response following IVH. The production of new neurons that can survive and integrate is a long-term goal. Mesenchymal stem cells (MSC) have powerful immune-modulating abilities after stroke [118,119]. Intraventricular transplantation of MSCs derived from the umbilical cord has been shown to prevent the development of brain injury and hydrocephalus following IVH [120]. The mechanism of this prevention and repair is likely due to the treatments ability to impact inflammatory cytokine production. However, additional studies have highlighted the neurotropic factors secreted by MSCs which promote astrogliosis and myelination [121]. The optimal route of delivery of MSCs after IVH appears to be intravenously rather than directly into the ventricles of the brain [122]; despite the greater efficiency of ICV delivery, there was no improvement in treatment efficacy over intravenous delivery. MSC use after IVH has been thoroughly reviewed elsewhere [123].

Following IVH, a buildup of hyaluronan in white matter lesions has been linked to inhibition of oligodendrocyte precursors cell maturation and myelination. Hyaluronan is a glycosaminoglycan polymer that inhibits remyelination [124]. Vinukonda et al. identified the possibility of treating this buildup of hyaluronan with hyaluronidase [125]. They found that hyaluronidase treatment in a rabbit IVH model reduced inflammation, increased oligodendrocyte precursor cell maturation, and restored myelination in the white matter lesions. While no other studies have focused on the possibility of repairing IVH-induced damage via hyaluronidase, it is a promising new lead that warrants further investigation.

6. Conclusion

Intraventricular blood can be a devastating complication of cerebral hemorrhage in preterm infants to adults. As yet, there is no therapy that reduces IVH-induced neurological deficits. However, preclinical data has identified multiple potential mechanisms for reducing such damage and enhancing brain repair. It is essential that more research be devoted to addressing whether those therapeutic avenues merit being pursued into clinical trial.

7. Expert opinion

Intraventricular blood can have devastating effects in both preterm infants and in adults. As in all stroke, prevention would be the best therapeutic option. While there are preclinical GMH/IVH models that can be used to study prevention in neonates, a paucity of adult spontaneous ICH/IVH models limits research and such models are sorely needed. One approach to limit GMH/IVH is to enhance vessel stability. There have been great strides made in understanding barrier-genesis in the CNS and this may provide new therapeutic targets. A potential concern with this approach is that those pathways may have side effects on brain development either via the vasculature (e.g. angiogenesis) or on other types of brain cell. Detailed dissection of the signaling pathways may provide more focused therapies.
IVH induces both primary (physical, mass effect) and secondary injury. Much of the primary injury may occur acutely after the IVH making it more difficult to treat. Fibrinolytic trials are, in part, designed to reduce the hematoma size and reduce the mass effect. Because of safety concerns, the initiation of fibrinolysis is delayed and while this may reduce further mass effects (e.g. on CSF drainage) it may be too late to prevent initial damage. Reducing the time for initiating fibrinolysis, by combining systemic hemostatic therapy with ICV fibrinolysis, may be of benefit.

There is growing data that clot-derived factors have an important role in IVH-induced secondary injury. Such factors include hemoglobin, iron, thrombin, and fibrinogen/fibrin, although there should be greater examination of the role other potential factors. Some of these clot-derived factors have multiple actions in IVH that might hamper them being targeted therapeutically (e.g. the role of thrombin in hemostasis as well as injury). There needs to be a dissection of downstream pathways activated by clot-derived factors to help identify better therapeutic targets.

Targeting hemoglobin- and iron-induced toxicity is attractive. There is a current phase II clinical trial for deferoxamine in ICH (iDEF Trial; NCT02175225). If the clinical experience with deferoxamine in ICH trial is successful, the experience with that treatment may be extended to other forms of cerebral hemorrhage including IVH. It should be noted that expanding deferoxamine to preterm IVH will require special attention to potential side effects. For example, iron deficiency is associated with a variety of neurological problems and iron is required for oligodendrocyte maturation [126]. It is possible that limiting the duration of deferoxamine exposure and/or using different routes of administration (e.g. ICV or intranasal) may limit some side effects.

Multiple of the clot-derived factors have a role in inflammation and inflammation itself may be a therapeutic target. As with thrombin, however, inflammation may have beneficial (e.g. in repair) as well as detrimental actions, effects which vary with time after hemorrhage. This makes it a difficult target. There is an enormous amount of preclinical information on the role of inflammation in brain injury after cerebral ischemia that has led to multiple clinical trials without success. While there are multiple potential reasons, the complex role of inflammation in brain repair as well as brain injury may be a contributing factor.

A new unexpected role of inflammation in IVH is in regulating CSF secretion [55]. Activation of choroid plexus epithelial TLR4, probably by hemoglobin or heme, activates a signal transduction pathway that stimulates NKCC1 activity and CSF secretion, contributing to PPH. That pathway needs to be examined in patients, but it may be inhibitable at several different steps [55].

As well as targeting the actions of clot-derived factors, there is also the possibility of reducing their release from the hematoma. The resolution of the hematoma after IVH isn’t well studied, but altering the fate of the hematoma, phagocytosis versus erythrolysis, may have important consequences for brain injury by determining whether hemoglobin/iron is released extracellularly or within microglia/macrophages. In ICH, there is evidence that phagocytosis can be enhanced by peroxisome proliferator-activated receptor (PPAR)-γ agonists [127] or manipulating eat-me/ don’t eat me signals on erythrocytes [128]. Alternatively, it may be possible to slow erythrolysis by targeting the complement cascade. Thus, the hematoma as well as neural tissue may be a therapeutic target, an understudied area.

There is the possibility of a combination therapy for reducing IVH-induced brain injury. There is evidence in adults that fibrinolytic therapy reduces IVH-induced mortality although it didn’t improve overall neurological outcome [6]. Fibrinolytic therapy does not fully remove the hematoma and perhaps the combination of that therapy with one targeting clot-mediated brain injury or one targeting brain repair might have greater efficacy.

Progress is being made in identifying therapeutic targets for IVH. As with other forms of stroke, the big hurdle is translating that knowledge to the clinic.

Acknowledgments

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Funding

This work was supported by the National Institutes of Health grants [NS073595, NS079157, NS090925, NS091545, NS093399 and NS096917].

Declaration of interest

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Papers of special note have been highlighted as either of interest (∗) or of considerable interest (∗∗) to readers.

This paper describes an important model of intraventricular hemorrhage due to hemorrhagic hydrocephalus.


**Preclinical evidence that hematoma-derived factors including iron contribute to post hemorrhagic hydrocephalus.**


**This paper describes an important model of intraventricular hemorrhage in premature rabbit pups.**


**Intraventricular hemorrhage is a consequence of intracerebral hemorrhage and this paper reviews mechanisms of injury and therapeutic targets in the latter.**
This study provides evidence that intraventricular hemorrhage stimulates CSF secretion contributing to post-hemorrhagic hydrocephalus and elucidates underlying mechanisms.


• **Current state of knowledge of choroid plexus ion and water transport.**


