Intraventricular Hemorrhage and White Matter Injury in Preclinical and Clinical Studies

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Education Gaps

1. Clinicians should be aware of the lack of effective postnatal interventions for the prevention and treatment of germinal matrix–intraventricular hemorrhage.
2. Physician-scientists should design studies evaluating prevention and treatment of germinal matrix–intraventricular hemorrhage in appropriate animal models.

Abstract

Germinal matrix–intraventricular hemorrhage (IVH) occurs in nearly half of infants born at less than 26 weeks' gestation. Up to 50% of survivors with IVH develop cerebral palsy, cognitive deficits, behavioral disorders, posthemorrhagic ventricular dilatation, or a combination of these sequelae. After the initial bleeding and the primary brain injury, inflammation and secondary brain injury might lead to periventricular leukomalacia or diffuse white matter injury. Potential factors that are involved include microglia and astrocyte activation, degradation of blood components with release of "toxic" products, infiltration of the brain by systemic immune cells, death of neuronal and glial cells, and arrest of preoligodendrocyte maturation. In addition, impairment of the blood-brain barrier may play a major role in the pathophysiology. A wide range of animal models has been used to explore causes and mechanisms leading to IVH-induced brain injury. Preclinical studies have identified potential targets for enhancing brain repair. However, little has been elucidated about the effectiveness of potential interventions in clinical studies. A systematic review of available preclinical and clinical studies might help identify research gaps and which types of interventions may be prioritized. Future trials should report clinically robust and long-term outcomes after IVH.

Objectives After completing this article, readers should be able to:

1. Recognize the multiple causes and mechanisms leading to germinal matrix–intraventricular hemorrhage.

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ABBREVIATIONS

A1M	alfa-1-microglobulin
AQP1	aquaporin 1
BBB	blood-brain barrier
CPE	choroid plexus epithelium
CSF	cerebrospinal fluid
DFX	deferoxamine
GM	germinal matrix
GMH	germinal matrix hemorrhage
hA1M	human plasma alfa-1-microglobulin
Hb	hemoglobin
Hp	haptoglobin
ICH	intracranial hemorrhage
IGF-1	insulinlike growth factor 1
IVH	intraventricular hemorrhage
MMP	matrix metalloproteinase
MSC	mesenchymal stem cell
NKCC	Na/K/Cl cotransporter
PAR	protease-activated receptor
PHVD	posthemorrhagic ventricular dilatation
rA1M	recombinant human alfa-1-microglobulin
rEPO	recombinant erythropoietin
SPAK	Ste20-type stress kinase
TGF-β	transforming growth factor β
TLR	Toll-like receptor
VEGF	vascular endothelial growth factor
WM	white matter

2. Describe the main effects of different interventions for the prevention and treatment of germinal matrix–intraventricular hemorrhage observed in preclinical and clinical studies.

INTRODUCTION

Intraventricular hemorrhage (IVH) continues to be a serious problem worldwide despite the progress of perinatal/neonatal medicine. Approximately one-third of preterm infants are affected by IVH, and as many as 45% of infants born at less than 26 weeks' gestation are affected. (1)(2) It has been reported that up to 50% of survivors with IVH develop cerebral palsy, cognitive deficits, behavioral disorders, posthemorrhagic ventricular dilatation (PHVD), or a combination of these outcomes. (3) Indeed, a meta-analysis suggests that even a germinal matrix hemorrhage (GMH) without parenchymal damage may affect long-term neurodevelopmental outcome. (4)

The early pathophysiological response associated with a GM-IVH induces the primary brain injury, followed by inflammation and secondary brain injury such as periventricular leukomalacia or diffuse white matter (WM) injury. The exact mechanisms of secondary brain injury after germinal matrix (GM)–IVH remain unknown. Several factors are involved, including microglia and astrocyte activation, degradation of blood components with release of "toxic" products, infiltration of the brain by systemic immune cells, death of neuronal and glial cells, and arrest of preoligodendrocyte maturation. Altogether, these events are associated with dysfunction of the blood-brain barrier (BBB). It is important to understand the possible mechanisms and triggers involved in the development of GM-IVH to identify possible approaches for prevention. Furthermore, understanding GM-IVH–induced secondary brain injury is a key factor for the development of efficient treatment strategies.

IVH MODELING

Animal Models

GM-IVH is a complex and multifactorial disease whose pathogenesis is not completely understood. Hemorrhage originates in the GM, and with further rupture of the ventricular ependyma, it may evolve into an IVH. (5) Due to its complexity, a variety of animal models of IVH have been used, involving different animal species, including mice, rats, rabbits, cats, dogs, sheep, pigs, and primates (Table 1). Most of the animal studies on IVH have focused on treatment rather than on prevention. The paucity of studies addressing prevention may be due to the fact that prematurity per se is such a strong risk factor for GM-IVH and prevention of preterm birth remains an unsolved issue. Most of the animal models described herein represent a mature systemic physiology coupled with a brain maturity that varies from extremely preterm to term compared with that of the human. The absence of actual preterm birth in these models disregards the interaction between important aspects of immaturity, such as trophic deprivation and coagulation impairment, and GM-IVH in the immature brain. With this central shortcoming in mind, these models still provide important information concerning IVH development, IVH-induced secondary brain injury, and possible treatment strategies.

One of the most common methods of inducing IVH in animals is by injecting centrifuged blood with an elevated hematocrit level into the ventricular space. This model has been used in rats, mice, and piglets, (6)(7)(8)(9)(10) with IVH developing in up to 70% of animals. This model can help investigate the role of blood components in PHVD formation and in WM damage. However, several limitations are present in these models. Postnatal day 7 in rodents and term age in piglets are considered appropriate time points for perinatal injury studies because the brain growth velocity is at its peak. (25) However, other organs are mature, which disregards important aspects of the clinical situation of preterm birth per se. Second, neither rodents nor piglets bleed spontaneously in the GM, suggesting that the inherent fragility of the GM vasculature is not present in these species. (26) Third, because the GM vasculature is intact, small periventricular infarctions, as observed in humans with periventricular hemorrhagic infarction, do not occur. (19)(26) In addition, corticospinal tract myelination is relatively advanced in rodents and piglets at these ages of brain maturation, rendering them more resistant to injury. (25)

Another commonly applied way of inducing intracerebral hemorrhage in rodent models aiming to mimic preterm human IVH is by performing stereotactic injection of collagenase. Collagenase is a proteolytic intracellular enzyme that catalyzes the hydrolysis of collagen. Because collagen is a fundamental component of the basal lamina of blood vessels, administration of collagenase into the brain

TABLE 1. **Most Commonly Used Animal Models of GM-IVH**

MODEL OF GM-IVH INDUCTION	ANIMAL	CLINICAL COMPARISON	MECHANISMS INVESTIGATED	REF. NO.
Centrifuged blood with high hematocrit, intracerebroventricular injection	Rodents Piglets	Brain development is comparable with gestational week 32 in human preterm newborn, myelination is advanced, other organ systems are mature	Role of blood components and erythrocyte lysis, immune response, integrity of BBB, neural-vascular unit damage	6-10
Collagenase injection into GM	Rodents	Brain development is comparable with gestational week 32 in human preterm newborn, myelination is advanced, other organ systems are mature	Brain morphology Neurodevelopmental outcome	11-15
Hypercarbia, hypertension, hypotension followed by rapid volume expansion	Dogs	GM layer is comparable with preterm humans, but the brain is overall mature, differences in cerebral perfusion	Role of cerebral blood fluctuations as causative mechanism	16-18
Intraperitoneal injection of glycerol to induce hyperosmolality	Preterm rabbits	Brain development corresponds to gestational week 24-25 in humans and cerebellum to gestational week 22-23 in humans, GM fragility is comparable with human preterm newborns, organ systems are immature	Role of extracellular hemoglobin and its metabolites, neuroinflammation, myelination, vascular fragility, neurocognitive and morphologic changes	19-21
Genetic models	Knockout rodents	Knockout mice embryos, mortality is nearly 90% shortly after birth, still challenging to create a model of spontaneous GM-IVH	Overexpression of VEGF, semidominant mutation of procollagen type IV α1, lack of αv integrin	22-24

BBB=blood-brain barrier, GM-IVH=germinal matrix–intraventricular hemorrhage, VEGF=vascular endothelial growth factor.

dissolves the extracellular matrix surrounding the capillaries, thus opening the BBB, and leads to intracranial bleeding. (11)(12)(13) In the collagenase model, neurocognition seems to be affected in a manner comparable with human infants. Moreover, similar to human infants, brain volumes are reduced after bleeding. (14)(15) However, it is known that collagenase induces a notable inflammatory response at the injection site that might affect the findings. (14) Unfortunately, data on morphologic changes in this particular model are lacking. A major disadvantage with this model is that the localization of the induced bleeding in this model does not correspond to that of preterm GM-IVH, with hemorrhages mainly being induced in periventricular tissue and basal ganglia. Although, this model is interesting and reproducible, it has obvious limitations.

It has been suggested in clinical studies that lack of cerebral blood flow autoregulation, rapid volume expansion, and hypercarbia may increase the risk of IVH occurrence. (5) Indeed, GM-IVH was induced in newborn beagle pups by provoking circulatory hypotension followed by rapid volume expansion. This model has led to the understanding that blood flow fluctuations are important in the pathogenesis of IVH, (16)(17)(18) which has influenced clinical practice.

Another animal model of IVH has been described in premature rabbit pups. (19)(20) Rabbit pups are delivered 3 to 4 days before term (full term = 32 days), and IVH is induced by intravascular hyperosmolarity via a postnatal intraperitoneal injection of glycerol. Of note, up to 10% of premature pups bleed spontaneously in the ventricles. (19) Moreover, the animals do exhibit signs of respiratory distress syndrome, as evident by tachypnea and intercostal and subcostal retractions, but do not require interventions supporting respiratory function. Brain development at gestational day 29 in the rabbit corresponds to approximately gestational week 24 or 25 in human infants for cerebrum formation and to week 22 or 23 for cerebellum formation. (19) Altogether, the rabbit glycerol model presents essential similarities to IVH in human preterm infants and includes immaturity of other organs as well. In this animal model, both behavioral deficits and morphologic changes have been described, such as neuroinflammation, periventricular cell death, preoligodendrocyte death, arrest of myelination, and

axonal damage. (19) It has been shown that extracellular hemoglobin (Hb) plays a crucial role in WM damage as it spreads widely across the WM tracts of the brain. (21)(27) Extracellular Hb and its metabolites may lead to a chronic persisting inflammation with long-term consequences for WM development after IVH.

All the animal IVH models reported previously herein lead to the development of PHVD.

Some genetic models of IVH have also been developed. In a spontaneous GM-IVH transgenic mouse embryo model, vascular endothelial growth factor (VEGF) overexpression is induced specifically in the GM via the tetracycline regulatory system. (22) VEGF is a central factor in angiogenesis, and its overexpression in the GM leads to an outgrowth of weak vasculature that is prone to rupture. In this model, there was a 90% incidence of spontaneous intracranial hemorrhage (ICH) that extended into the ventricles; however, a critical disadvantage of this model is the high mortality (approximately 80% of embryos die before birth). In another genetic mutation model characterized by a semidominant mutation in procollagen type IV alpha 1, ICH and death occurred within 1 day of birth. (23) McCarty and colleagues (24) found that the mice lacking αv integrin developed ICH in utero and died soon after birth. Thus, genetic models of GM-IVH illustrate important aspects of IVH evolution but present limitations for the study of short- and long-term outcomes.

In vitro Models

There are currently no in vitro models that resemble IVH. Nevertheless, different cell culture techniques have been used to investigate the effect of clot-derived factors, such as thrombin, Hb and its metabolites, iron, and thrombin.

PREVENTION

The major risk factor for the development of GM-IVH in humans is premature birth because the GM involutes almost completely by gestational week 36 in human infants. (5) Preventing preterm birth is a complicated area of research and has been reviewed elsewhere. (28) However, due to a significant improvement in perinatal medicine, the survival of extremely preterm infants is increasing worldwide. Nonetheless, the incidence of IVH has remained nearly the same during the past 2 decades despite increased absolute numbers of IVH. (1)(2)(29)

The GM vasculature exhibits unique characteristics explaining its propensity for rupture. The endothelial proliferative rate in the GM is extremely high, coupled with a high expression of VEGF and angiopoietin-2. (5) In addition, the GM, compared with other cerebrovascular regions, has a lower number of pericytes, decreased presence of transforming growth factor β (TGF-β), and lower expression of fibronectin-1 in basal lamina. (5) In line with these findings, prenatal administration of angiogenic inhibitors in the preterm rabbit model decreased the incidence of GMH. (30) Thus, by suppressing proliferation and increasing vascular maturation in the GM, the incidence of GMH may be reduced in preterm infants. Interestingly, treatment with insulinlike growth factor 1 (IGF-1) in complex with IGF binding protein-3 seemed to decrease the rate of severe IVH in extremely preterm infants. (31) These effects may relate to a maturational effect of administered IGF-1/IGFBP-3 on GM vasculature.

As mentioned previously herein, extremely preterm infants undergo rapid changes in cerebral blood flow after birth, and this has been reported in the beagle IVH model. (16)(17)(18) It has been suggested that care strategies initiated in the delivery room aiming to reduce hemodynamic fluctuations might reduce the risk of GM-IVH. Delayed cord clamping is another intervention in the immediate postnatal period that has been shown to have an effect on the incidence of GM-IVH, reducing its occurring risk. (32) One could speculate that an increased circulating blood volume in infants receiving delayed cord clamping has a stabilizing effect on cerebral blood flow; in addition, delayed clamping may have an enhancing effect on the BBB by transfusing stem cells, growth factors, exosomes, microvesicles, and anti-inflammatory substances. Finally, delayed cord clamping may improve hemostasis by transfusion of coagulation factors, platelets, and Hb scavengers. Animal studies of delayed cord clamping may elucidate the mechanisms involved in diminishing the risk of IVH.

GM-IVH–INDUCED BRAIN INJURY

Brain injury after IVH may be divided into 2 different phases: the primary injury, when actual bleeding occurs, and the secondary injury, which is induced by neuroinflammation. Most of the preclinical research studies have examined the secondary brain injury, with a particular focus on WM injury.

Primary Injury

Physical Effect of IVH. There are at least 3 physical effects of IVH: displacement of neural tissue (mass effect), increased intracranial pressure, and blockage of cerebrospinal fluid (CSF) pathways. Once bleeding occurs there is a mechanical effect on the ventricular wall, including direct stretching of the wall and the periventricular tissue along the ventricles. There are no preclinical data available addressing the

physical impact of the space-occupying hematoma. However, neurophysiological assessments in preterm infants with IVH who are subsequently diagnosed as having PHVD have demonstrated an early alteration in visual evoked potentials and amplitude-integrated electroencephalography, which normalize as soon as effective PHVD treatment is started (evacuation of CSF). (33)(34)

Another potential physical effect of IVH is the transient blockage of the CSF drainage pathway, (35) either in the ventricular system or at the CSF outflow sites (Fig 1). It is hypothesized that this blockage may contribute to the development of PHVD. Furthermore, a physical block by blood clots and outflow site fibrosis may lead to a further alteration of CSF homeostasis. A classical model of CSF dynamics postulates that the development of PHVD requires obstruction of CSF flow from the ventricles and/or impairment of the arachnoid granulations: these might be classified as a primary decrease in CSF reabsorption. However, this hypothesis, which is supported by limited experimental evidence, (36) neglects the potential role of increased CSF secretion in disease pathogenesis. (37) Indeed, hypersecretion of CSF after IVH seems to depend on Toll-like receptor (TLR) 4 activation and nuclear factor kB–dependent inflammatory response in the choroid plexus epithelium (CPE). This leads to activation of Ste20-type stress kinase (SPAK) and further phosphorylation and activation of Na/K/Cl cotransporter (NKCC) 1 at the apical surface of the CPE (Fig 1). (37) In fact, genetic depletion of TLR4 or SPAK restores hyperactive CSF secretion rates and improves PHVD symptoms. Similarly, treatment with inhibitors of TLR4–nuclear factor kB signaling or the SPAK–NKCC1 complex reduces the hypersecretion of CSF, thereby diminishing by PHVD and its related symptoms. (37) Further studies targeting TRL4 and SPAK pathways are warranted.

Mass Effect. Clearing the hematoma after IVH may reduce the mass effect and be useful for decreasing levels of toxic substances in the brain. (38)(39)(40)(41)(42) Fibrinolytic therapy does not reduce death or need for a ventriculoperitoneal shunt. (43) Although fibrinolytic therapy has been shown to increase the incidence of secondary hemorrhage, the long-term DRIFT (drainage, irrigation, and fibrinolytic therapy) study at age 2 years showed reduction of severe disability or death and improvement of cognitive function in preterm infants with severe IVH and PHVD. (43) To our knowledge, there are no ongoing preclinical studies in newborn IVH models that examine the efficacy and safety of fibrinolytic therapy.

Alterations in CSF Homeostasis. Hyperproduction of CSF occurs after IVH. Approximately 80% of CSF is secreted by CPE, (44)(45)(46) whereas the remaining

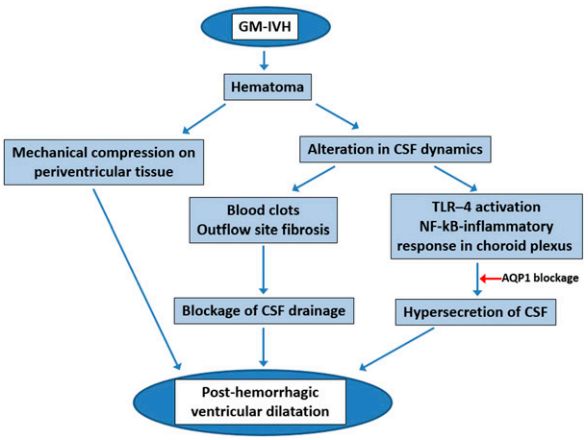

Figure 1. Impact of hematoma in germinal matrix–intraventricular hemorrhage (GM-IVH). AQP1=aquaporin 1, CSF=cerebrospinal fluid, NF-kB=nuclear factor kB, TLR=Toll-like receptor.

20% originates from the brain interstitial fluid and indirectly from the BBB. (47) The estimated surface area of the human BBB (20 m^2) (48) exceeds the surface area of the CPE, which is estimated to be 0.02 m^2 (49); accounting for the surface extension of microvilli, the CPE surface may maximally extend to 5% of the BBB area. Nonetheless, because the BBB has a low permeability to ions and water, the expected high transport rate of CPE might be sufficient to explain CSF production. However, there are some controversies about this central paradigm, as discussed elsewhere. (46)(50)(51)(52)(53)

One of the possible treatment strategies for PHVD could be normalization of the CSF secretion rate. The CPE contains tight junctions and a wide range of ion transporters and water channels (aquaporin 1 [AQP1]), which can be altered. This was the rationale to assess the effect of 2 diuretics in human clinical trials of IVH: a combination of acetazolamide (a carbonic anhydrase inhibitor) and furosemide (an Na/Cl cotransport inhibitor). This therapy was effective for reducing CSF secretion, but it did not decrease the need for ventriculoperitoneal shunt placement and actually increased the neurologic morbidity. (54)(55)(56) There are no preclinical studies on the use of these diuretics in animals with PHVD, except for a recent investigation demonstrating mild reduction of CSF production in the rat with PHVD. (37) AQP1 is extremely important for water transcellular transport, and AQP1 knockout mice have an approximately 80% reduction in water permeability from CPE. (57) Moreover, there is a concomitant decrease in the CSF secretion rate by 35% by CPE, (57) which is nearly 50% of the total CSF secretion rate because CPE secretes only 70% to 80% of the CSF. It seems that AQP1 may have a central role in CSF formation; however, it is unclear to what extent AQP1 mediates the transepithelial water transport.

Surprisingly, there are not much data available on how IVH affects water transport in the choroid plexus. Sveinsdottir et al (58) demonstrated an increase in choroid plexus AQP1 protein expression in a preterm rabbit IVH model (Fig 1). More studies are needed to understand the possible role of AQP1 in PHVD development after IVH. It seems that targeting AQP1 may represent a possible treatment strategy.

Secondary Injury

Blood Components and Brain Injury. Figure 2 depicts an overall picture of the mechanisms involved in secondary brain damage after IVH and potential therapeutic targets.

A considerable amount of research in this area has indicated that secondary brain injury might be caused by blood components. Once IVH occurs, erythrocytes undergo hemolysis with release of extracellular Hb and its degradation products that are toxic to the immature brain. Moreover, other blood components (eg, coagulation factors) and immune cells can independently induce/aggravate brain damage. Studies on blood component–based mechanisms of secondary brain injury have led to the identification of potential therapeutic targets. The following section examines the mechanisms of blood-derived toxicity.

Hemolysis, Hb, and Its Degradation Products. After IVH in preterm infants, there is deposition of blood in the intraventricular space, followed by lysis of erythrocytes, resulting in a subsequent release of extracellular Hb into the CSF. (27) Extracellular Hb is highly reactive and rapidly oxidized from ferrous (Fe^{2+}, denoted oxyHb) to ferric (Fe^{3+}, denoted metHb) Hb, (27)(59) which readily releases the heme group. (60) Free heme, iron, and oxygen are highly redox reactive and can damage lipids, proteins, and DNA through oxidative modification, cross-linking, and fragmentation. (60) Heme binds to lipids of cell membranes, leading to toxic cytolytic effects through both oxidative and nonoxidative mechanisms. (61) In addition to its redox-related effects, heme has been described to act as a damage-associated molecular pattern molecule that triggers TLR-mediated proinflammatory damaging pathways. (62)(63)(64) Moreover, extracellular Hb causes structural damage of the CPE as soon as 24 hours after IVH in preterm rabbit pups and results in severe cellular disintegration with loss of normal villous morphology and signs of cellular apoptosis and necrosis 72 hours after IVH. (27)(65) Furthermore, it has been shown in animal studies that extracellular Hb activates the inflammatory cascade, resulting in elevation of tumor necrosis factor α in the CSF. (27) The elevation of tumor necrosis factor α IVH was identified in preterm human infants as well. (27)

Iron is another product that is released in abundance after IVH and erythrocyte lysis and is involved in the

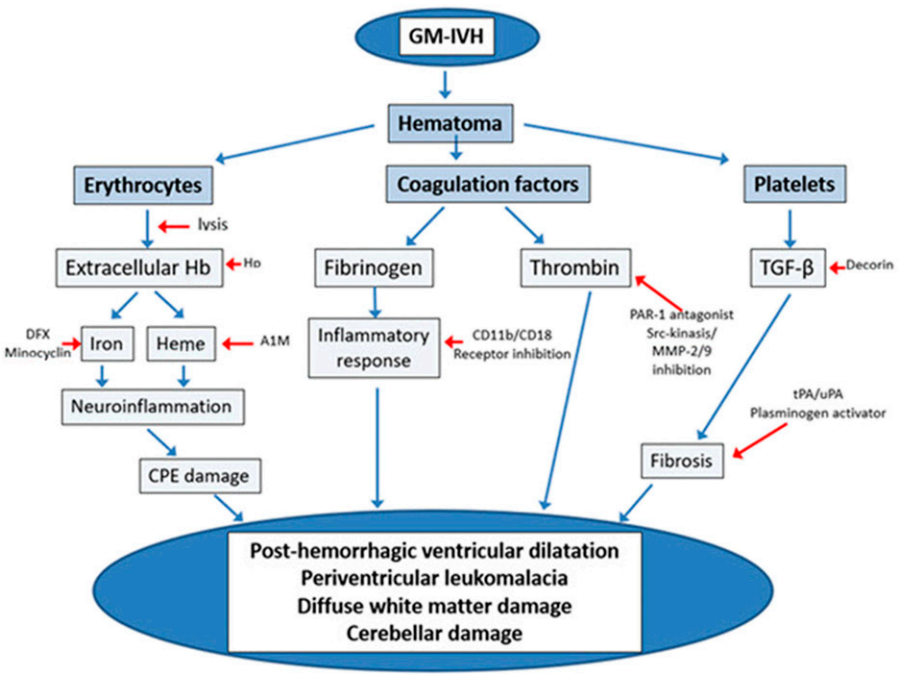

Figure 2. Pathological mechanisms underlying the formation of posthemorrhagic ventricular dilatation, periventricular leukomalacia, and white matter and cerebellar damage. A1M=alfa-1-microglobulin, CPE=choroid plexus epithelium, DFX=deferoxamine, Hb=hemoglobin, Hp=haptoglobin, MMP=matrix metalloproteinase, PAR=protease-activated receptor, TGF-β=transforming growth factor β, tPA/uPA=tissue and urokinase plasminogen activator.

secondary brain damage during IVH and PHVD development. (66) Non–protein-bound iron and ferritin deposit in ependymal lining after neonatal IVH. (67)(68) Interestingly, non–protein bound iron was found in the CSF of preterm infants with PHVD. (69) In animal IVH rat models, iron deposition after IVH already occurs in the first day, and upregulation of brain ferritin lasts for several weeks. (66) The deposition of iron along the ventricular ependyma may lead to denudation areas in the lining, facilitating the movement of extracellular Hb deeper into the periventricular tissue and resulting in diffuse WM injury. Indeed, a wide distribution of extracellular Hb was found in both adjacent periventricular tissue and deeper in WM tracts involving the corpus callosum, corona radiate, subthalamic regions, and hippocampus in the preterm rabbit pup IVH model. (21) Periventricular tissue is characterized by a high presence of preoligodendrocytes, which progressively differentiate to mature oligodendrocytes. This process is extremely important for normal WM development, and alterations may lead to diffuse WM damage and periventricular leukomalacia that are found in premature infants. This process is particularly active between weeks 23 and 35 of gestation. (70)(71) Preoligodendrocytes are highly vulnerable to oxidative stress, inflammation, and hypoxic-ischemic events. (70)(71) In addition to periventricular distribution, extravasation of red blood cells and Hb into the CSF have been shown to result in deposition of Hb metabolites on the cerebellar surface, leading to alteration of normal development of the cerebellar cortex and its related functions. (72)(73)(74) Furthermore, the causal involvement of extracellular Hb in compromised cerebellar neuronal progenitor proliferation and delayed Purkinje cell maturation after IVH has been reported. (74)

Hemolysis, Hb, and Its Degradation Products—Possible Treatment Targets. As described previously herein, data suggest that extracellular Hb and its degradation products play a central role in secondary brain damage after IVH. Thus, targeting extracellular Hb and its metabolites may represent valuable treatment strategies. Physiologically, several endogenous systems are involved in the neutralization of extracellular Hb and its degradation products, such as haptoglobin (Hp), high- and low-density lipoproteins, serum albumin, and hemopexin.

Hp is crucial to prevent extra-erythrocytic Hb-induced injury because of its high binding affinity for extracellular Hb. Hp is synthetized mainly in the liver and is an acute phase protein. (75) The key role of Hp is to clear the intravascular extracellular Hb. Hp binds to extracellular Hb, forming a stable Hb–Hp complex, which then funnels the Hb molecules for intracellular endocytosis. (76)(77) Intracellularly, the enzyme heme oxygenase 1 breaks down heme to bilirubin and carbon monoxide, which have antioxidant and vasodilatory properties. (78) By forming a tight complex with extracellular Hb, Hp stabilizes and shields heme iron in the hydrophobic pocket of Hb, thereby preventing its cytotoxic and pro-oxidative damage. (79) Therefore, the elimination of extracellular Hb from the extracellular environment by the formation of Hb-Hp complexes reduces the interaction of extracellular Hb with the brain's innate immune system cells signal-transducing receptors and, thereby, diminishes exposure to iron overload and to heme-induced toxicity. After intracerebral hemorrhage, oligodendroglial cells start to produce Hp, (80) thereby protecting axons from damage imposed by hemolytic products. Mice overexpressing Hp showed better preservation of axonal integrity after ICH. (80) Importantly, Hp-overexpressing oligodendrocytes in culture showed reduced loss of myelin basic protein on exposure to hemolytic products compared with oligodendroglia from Hp-deficient mice. (80) This recent finding is of extreme importance considering the role of oligodendrocytes in diffuse WM injury after IVH, thus representing a potential treatment strategy. In fact, Hp knockout mice had a worse neurologic outcome after ICH, whereas overexpression of Hp was neuroprotective. (80)

However, the local production levels of Hp may be insufficient to fully scavenge extracellular Hb. (81) Galea et al (81) demonstrated that after adult subarachnoid hemorrhage, the Hb-Hp system was quickly saturated with a residual inability to handle extracellular Hb, indicating an insufficient Hb scavenging capacity in the brain. Indeed, in premature rabbit pups that underwent intraventricular administration of Hp, there was a partial reduction in the Purkinje cell maturational arrest caused by IVH, which in turn related to decreased impairment of the proliferative portion of the external granular layer after IVH. (74)

Despite multiple positive effects of Hp on preservation of secondary brain damage after IVH, a recent in vitro study showed that Hb could be toxic to the neurons in an Hp-dependent manner because of a lack of iron-sequestering systems that may lead to iron overload intracellularly. (82) Intracellular iron is free to react with available hydrogen peroxide to form radical oxygen species, resulting in induction of apoptotic cascade and cell death. (82) Additional well-designed studies are needed to detect possible beneficial and detrimental effects of Hb scavenging through administration of Hp.

Deferoxamine (DFX) is a ferric-iron chelator that is used clinically for acute iron poisoning, systemic iron overload, and hemochromatosis. It has been suggested that DFX may attenuate the brain injury and improve neurologic outcome after IVH/ICH. (83) However, the authors of the systematic

review on DFX effects in animal IVH/ICH models raise concerns about the low quality of the study and possible publication bias, concluding that DFX neuroprotective effects need to be further assessed. (83) Beneficial effects of DFX after IVH/ICH include reduction in iron overload–induced edema, (84) cell death and neuronal degeneration in periventricular tissue, (8)(66)(85)(86) hippocampal degeneration, (8) and inflammation. (84)(85)(87) One of the phase 2 clinical trials in adults with ICH treated with either DFX or placebo was suspended due to an increased incidence of acute respiratory distress syndrome. (88) The authors decreased the dosage of DFX and conducted a double-blind multicenter phase 2 study in adult ICH. (89) The treatment was considered to be safe, but it did not improve neurologic outcomes of patients with ICH. Longer follow-up is needed. Another ongoing phase 2 study is registered to assess the effects of DFX and xingnaojing injection treatment in intracerebral hemorrhage. (90)

Another potential treatment strategy might be minocycline, which is a tetracycline antibiotic used to treat infections, rheumatoid arthritis, and acne. It has good BBB penetration, chelates iron, inhibits microglia, and reduces apoptosis and inflammation. (91)(92)(93) Minocycline may diminish iron accumulation in an experimental GM-IVH model, resulting in alleviation of brain edema, PHVD, and cell death. (94) Minocycline is thought to suppress ferritin upregulation after hemorrhage by acting through the cannabinoid receptor 2, inhibiting the inflammatory cascade. (94) In a small, clinical phase 2 study in an adult population after ICH, it has been shown that minocycline is safe; however, no difference in inflammation markers (matrix metalloproteinase [MMP]-9, interleukin-6, iron, ferritin, total iron-binding capacity), ICH volume, or perihematomal edema in patients treated with minocycline were detected compared with the control group. (95) An ongoing phase 2 study on the effects of minocycline after ICH in adults has been registered. (96)

Another potential treatment target strategy for IVH is the heme and free radical scavenger alfa-1-microglobulin (A1M). A1M has been demonstrated to be a potent tissue-protective protein that mediates its effects through heme binding, reductase activity, radical scavenging, and binding to mitochondria. (97)(98) Mitochondrial uptake of A1M in the early stages of cell death results in inhibition of heme- and reactive oxygen species–induced mitochondrial swelling. (99) A recombinant human A1M (rA1M) has been developed (100)(101) and has been shown to be functionally equivalent to endogenous A1M derived from human plasma (hA1M). (102)(103) The brain has a minimal production and systemic distribution of endogenous A1M, reflected in the trace quantities of the protein present in CSF compared with other body fluids: CSF, 0.0423 mg/L; serum, 44.2 mg/L; synovial fluid, 20.8 mg/L; ascites, 28.7 mg/L; pleural effusion, 21.5 mg/L; and amniotic fluid, 0.0027 mg/L. (104) Interestingly, an increase in the concentration of endogenous A1M in CSF after ICH has been documented in adult human patients. The increase was associated with serum infiltration as a result of BBB disruption; however, local production could not be excluded. (105)

Based on the pathophysiological mechanisms described for brain injury after IVH, exogenously administered A1M seems to be a promising treatment alternative. In a preterm rabbit pup IVH study, exogeneously administered rA1M had a wide distribution across brain and cerebellar WM. (106) Moreover, some functional studies detected that hA1M preserved mitochondrial structure and arrested the fusion of mitochondria. (106) Indeed, hA1M administration significantly reduced cellular activation, inflammatory response, and tissue injury, suggesting that administration of hA1M blocks the toxic reactions of extracellular Hb metabolites. (106) More studies are needed to understand the mechanism of action, safety, and beneficial effects of rA1M.

Coagulation Components. Besides erythrocytes, plasma components play a role in IVH-induced secondary brain injury. In particular, some of the elements of the coagulation system seem to be involved, including prothrombin/thrombin (factor IIa). Thrombin is formed after the cleavage of prothrombin in the clotting process, which is upregulated during hemorrhage. Thrombin is essential to convert soluble fibrinogen into insoluble fibrin to prevent bleeding. Immediate production of thrombin in the brain occurs after a cerebral hemorrhage or BBB disruption due to brain injury. (107) Indeed, several in vivo (108)(109)(110) and in vitro (111)(112) studies indicate that a high brain concentration of thrombin may be deleterious. It has been shown that a high concentration of thrombin is involved in brain edema formation and contributes to ischemic brain injury. (113) Furthermore, in high concentrations, thrombin induced neuronal and astrocyte cell death. (112)(113) However, a low brain concentration of thrombin (50–100 nM) is neuroprotective. (111)(114) Such a dichotomy of thrombin in the brain may reflect its multiple functions. It enhances the synthesis and secretion of nerve growth factor in glial cells, modulates neurite outgrowth, stimulates astrocyte proliferation, and modulates the cytoskeleton of endothelial cells. (115) In addition, it potentiates *N*-methyl-D-aspartate receptor function (116) and activates rodent microglia in vitro. (117)

Most thrombin functions are mediated through protease-activated receptor (PAR) 1, PAR-3, and PAR-4. (118) PAR-1

activation after IVH results in ependymal wall damage, which may contribute to the development of PHVD. (119) Activated thrombin–PAR-1 system results in activation of the Src family of kinases, which are responsible for the phosphorylation of metalloproteinases, in particular MMP-9, which have been shown to lead to disruption of BBB and induce apoptosis. (120)(121)(122) By using PAR-1 antagonists, injury to the ependymal wall was reduced after IVH. (119) Furthermore, a nonspecific Src family kinase inhibitor PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d] pyrimidine) stops the development of brain edema and BBB disruption after IVH. (123) Inhibition of MMP-2 and MMP-9 by GM6001 resulted in reduction of brain edema and decreased inflammatory cell infiltration after ICH. (122)

Thrombin-induced inflammation has been linked to TGF-β. (124) It is believed that TGF-β is released into the CSF after platelet extravasation during IVH, and it may play a role in the formation of obstructive hydrocephalus. (125) In juvenile communicating hydrocephalus clinical studies, it has been shown that decorin, a TGF-β antagonist, could reverse ventriculomegaly and WM injury. (126)(127) Nevertheless, further research is necessary to fully elucidate the mechanism by which TGF-β is involved in hydrocephalus after IVH.

Fibrinogen (factor I) may also be involved in secondary IVH-induced brain injury. Despite the essential role in conversion of fibrinogen to fibrin for hemostasis, the formed clots may result in obstruction of the normal circulation of CSF. Furthermore, it has been shown that the extravascular presence of fibrinogen is a powerful inflammatory response trigger, activating microglia via the CD11b/CD18 receptor, (128) which may aggravate brain injury.

Although coagulation system elements may play an important role in IVH-induced secondary brain injury, targeting them therapeutically is complicated due to their vital role in hemostasis. The therapeutic window may need to focus on targeting downstream mediators (eg, through thrombin-mediated PAR-1 activation or targeting fibrinogen-mediated microglial activation).

Other blood components may also contribute to IVH-induced secondary brain damage. Recently it has been shown that lysophosphatidic acid, present in serum, can impair ependymal integrity. (129) Lysophosphatidic acid is produced and released by activated platelets. (130) Of note, the role of platelets in IVH-induced secondary brain injury has received very little attention.

PRECLINICAL FINDINGS ON REPAIR OF INJURY

In addition to the preclinical studies addressing the possible reduction or arrest of IVH-induced secondary brain injury, other studies have explored the possibility of augmenting brain repair of injured tissue. The main strategies have focused on enhancing neurogenesis, stem cell therapy, and reversal of hyaluronan build-up (Table 2).

Recombinant erythropoietin (rEPO), mainly known for stimulating red blood cell production, displays neuroprotective capabilities and might restore the damage in the GM. (153) This is of particular interest because IVH originates from the GM, which is a source of arising neurons and glial cells in the developing brain. In studies on neonatal hypoxic ischemia, rEPO administration may enhance neurogenesis and oligodendrogenesis (131)(132) and limit inflammation and oxidative stress–induced injury. (133)(134) Administration of rEPO combined with melatonin may reduce ventriculomegaly in the rat pup with IVH. (135) Moreover, the microstructural integrity of WM and gray matter, ultrastructural integrity of ependymal motile cilia, and periventricular lysophosphatidic acid yes-associated protein were restored in treated pups. (135) A large prospective clinical trial of rEPO treatment in preterm infants did not show any benefit on IVH or mortality rates. (136) A similar trial is aiming to investigate the same research question. (137) ↳ EPOREPAIR

Another potential treatment option for IVH might be melatonin, which acts as an antioxidant and free radical scavenger, (138)(139) inhibits free radical–associated red blood cell lysis, (140) decreases neuronal cell death, (141) and decreases hippocampal and nigrostriatal degeneration. (142) In a rat animal IVH model, treatment with melatonin improved neurobehavioral outcomes and reduced the level of brain atrophy. (143)

Stem cell therapies represent a promising treatment approach for IVH. Stem cells were first used to treat intracranial bleeding in adult rats in a collagenase-induced ICH model. (154) Subsequently, it has been shown that treatment with umbilical cord–derived mesenchymal stem cells (MSCs) in an animal ICH model resulted in nerve fiber remyelination, axonal regeneration, and improved neurologic recovery. (155) In a neonatal IVH rat pup model, MSC administration either intracerebrally or intravenously resulted in attenuation of PHVD, better myelination, and better performance on behavioral tests. (144) Indeed, further studies demonstrated that early MSC administration resulted in greater recovery from brain injury. (145) It is suggested that regenerative cells exert their therapeutic benefit through the release of paracrine effects. (156) They promote axon and dendrite growth by secreting mitogenic

TABLE 2. **Preclinical Findings and Ongoing Studies of IVH-Induced Damage Repair**

INTERVENTION	PRECLINICAL DATA	CLINICAL DATA	ONGOING STUDIES
Recombinant erythropoietin	Enhances neurogenesis and oligodendrogenesis, (131)(132) limits inflammation and oxidative stress–induced damage, (133)(134) reduces ventriculomegaly, attenuates microstructure of white and gray matter (135)	Safe, (136) did not decrease IVH incidence or mortality rates (136)	NCT02076373 (137): a randomized controlled trial to investigate the possible protective role of recombinant erythropoietin in preterm infants with IVH
Melatonin	Acts as antioxidant and free radical scavenger (138)(139); inhibits free radical–associated red blood cell lysis, (140) neuronal cell death, (141) hippocampal and nigrostriatal degeneration (142); improves neurobehavioral outcomes and reduces the level of brain atrophy (143)	Not available	Not available
Stem cell–based therapies	Attenuates PHVD, better myelination, better performance on behavioral tests, (144)(145) ameliorates inflammation, (146) enhances angiogenesis, regulates reactive oxygen species production, transfers organelles to injured cells (146)(147)(148)(149)	One phase 1 study (9 premature infants with IVH grade 4): no serious adverse effects or dose-limiting toxicities attributable to mesenchymal stem cell transplant; 5 of 9 infants required shunt derivation. (150) Follow-up assessment is ongoing (NCT02673788 [146]).	NCT02890953 (151): a phase 2 randomized controlled trial, preterm infants with IVH grade 3-4. Primary outcome: death or shunt operation; secondary outcomes: volume ratio of ventricle to whole brain in the brain MRI; death.
Hyalorinidase	Reduces inflammation, increases oligodendrocyte precursor cell maturation, restores myelination in white matter lesions (152)	Not available	Not available

IVH=intraventricular hemorrhage, MRI=magnetic resonance imaging, PHVD=posthemorrhagic ventricular dilatation.

growth factors (brain-derived neurotrophic factor, stromal cell–derived factor 1, and nerve growth factor) known to enhance proliferation, migration, and differentiation of native neuronal progenitor/stem cells. (147)(148)(149) Of note, cell-based therapies have been demonstrated to ameliorate inflammation (interleukin-1β, interferon-γ) by modulating the function of immune cells such as T and B cells, macrophages, and dendritic cells. (146) In addition, they may enhance angiogenesis (VEGF, IGF-1), regulate reactive oxygen species production, and even transfer organelles to injured cells. (146)(147)(148)(149) To date, 1 phase 1 study was performed on 9 preterm infants with a diagnosis of IVH grade 4; no serious adverse effects or dose-limiting toxicities attributable to MSC transplant were identified. (150) No infants died during the study period. Five of 9 infants required shunt derivation. (150) The follow-up assessment (2 years of age) is currently ongoing. (157) Moreover, a phase 2 trial is planned to randomize preterm infants with IVH grade 3-4 to direct intracerebroventricular injection of either MSCs or normal saline. (151)

After IVH, a formation of hyaluronan in WM lesions has been linked to inhibition of oligodendrocyte precursor cell maturation and myelination. Hyaluronan is a glycosaminoglycan polymer that inhibits remyelination. (158) In the preterm rabbit IVH model, hyaluronidase treatment reduced inflammation, increased oligodendrocyte precursor cell maturation, and restored myelination in the WM lesions. (152)

CONCLUSIONS

GM-IVH is a complex brain condition in the preterm newborn that may lead to long-term neurodevelopmental impairment. Currently, no clear-cut effective prevention or treatment is available for these infants. Preclinical data have identified potential mechanisms for reducing IVH-induced brain damage and enhancing brain repair. Systematic reviews of preclinical studies in adequate animal models and of randomized clinical trials that synthesize the body of evidence is important to highlight the research gaps that should be investigated.

American Board of Pediatrics Neonatal-Perinatal Content Specifications

- Know the risk factors for development, proposed mechanisms, clinical and laboratory features, and diagnosis of periventricular-intraventricular hemorrhage.
- Know the proposed prevention strategies, evolution, early complications, management, and long-term consequences of periventricular-intraventricular hemorrhage.
- Know the risk factors for development, proposed mechanisms, clinical and laboratory features, and diagnosis of intraparenchymal cysts/periventricular leukomalacia, and intraparenchymal echodensities.
- Know the pathogenesis, clinical and imaging features, diagnosis, management, and outcomes associated with perinatal cerebral and cerebellar infarction.

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1. Intraventricular hemorrhage (IVH) is a serious complication of prematurity and affects approximately one-third of preterm infants. The incidence of IVH increases at lower gestational ages. What is the incidence of IVH in infants born at less than 26 weeks' gestational age?
   A. 15%.
   B. 25%.
   C. 35%.
   D. 45%.
   E. 55%.

2. IVH can lead to significant long-term neurodevelopmental impairments. The pathophysiology of IVH is multifactorial and includes the unique features of the germinal matrix (GM) vasculature. Which of the following statements regarding the GM vasculature is CORRECT?
   A. The endothelial proliferative rate in GM is low.
   B. There is high expression of vascular endothelial growth factor in the GM endothelium.
   C. The GM has a lower number of pericytes compared with other brain regions.
   D. The levels of transforming growth factor are decreased.
   E. The basal lamina exhibit lower expression of fibronectin-1 compared with other brain regions.

3. Brain injury after IVH occurs in 2 phases. At the time of bleeding (primary phase), brain injury is due to the physical effects of IVH as well as alterations in cerebrospinal fluid (CSF) homeostasis. Which of the following statements regarding the impact of IVH on CSF homeostasis is INCORRECT?
   A. Outflow site fibrosis is a mechanism of CSF drainage blockage.
   B. Hypersecretion of CSF after IVH occurs in 50% of affected infants.
   C. Activation of Toll-like receptor 4 after IVH contributes to CSF hyperproduction.
   D. The inflammatory response in the choroid plexus epithelium results in activation of the Na/K/Cl cotransporter 1.
   E. Aquaporin 1, located in the choroid plexus epithelium, is an important channel regulating the CSF secretion rate.

4. Multiple mechanisms contribute to secondary brain injury after IVH. Which of the following statements regarding the secondary phase of brain injury after IVH is CORRECT?
   A. The presence of extracellular hemoglobin leads to the rapid oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), a highly redox reactive compound directly linked to lipid, protein, and DNA damage.
   B. Free heme can bind to lipids in the cell membrane, leading to toxic cytolytic effects through both oxidative and nonoxidative mechanisms.
   C. Extracellular hemoglobin has been shown to cause structural damage to the choroid plexus epithelium within 1 hour of injury.
   D. Loss of normal villous morphology with cellular apoptosis and necrosis can be seen within 72 hours of injury in preterm rabbits.
   E. Free heme has been shown to activate Toll-like receptor–mediated proinflammatory pathways.

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5. The promotion of brain repair after IVH is 1 of the therapeutic strategies currently under investigation. Which of the following statements regarding brain repair strategies in preclinical models is CORRECT?

A. The mechanisms of neuroprotection after recombinant erythropoietin administration include enhanced neurogenesis and oligodendrogenesis.
B. Melatonin acts as an antioxidant and free radical scavenger, and has been shown to decrease brain atrophy in a rat model of IVH.
C. Treatment with mesenchymal stem cells has been shown to decrease post-hemorrhagic ventricular dilatation in a rat model of IVH.
D. Mesenchymal stem cell administration has been shown to promote axon and dendrite growth.
E. Treatment with hyaluronidase limits inflammation and oxidative stress.