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Inflammation of the Choroid Plexus and Ependymal Layer of the Ventricle Following Intraventricular Hemorrhage

Philippe F. Simard,
Department of Neurosurgery, University of Maryland, School of Medicine, 22 S. Greene St., Suites S-12-D, Baltimore, MD 21201-1595, USA

Cigdem Tosun,
Department of Neurosurgery, University of Maryland, School of Medicine, 22 S. Greene St., Suites S-12-D, Baltimore, MD 21201-1595, USA

Ludmila Melnichenko,
Department of Neurosurgery, University of Maryland, School of Medicine, 22 S. Greene St., Suites S-12-D, Baltimore, MD 21201-1595, USA

Svetlana Ivanova,
Department of Neurosurgery, University of Maryland, School of Medicine, 22 S. Greene St., Suites S-12-D, Baltimore, MD 21201-1595, USA

Volodymyr Gerzanich, and
Department of Neurosurgery, University of Maryland, School of Medicine, 22 S. Greene St., Suites S-12-D, Baltimore, MD 21201-1595, USA

J. Marc Simard
Department of Neurosurgery, University of Maryland, School of Medicine, 22 S. Greene St., Suites S-12-D, Baltimore, MD 21201-1595, USA. Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201, USA. Department of Pathology, University of Maryland, School of Medicine, Baltimore, MD 21201, USA

J. Marc Simard: msimard@smail.umaryland.edu

Abstract

Intraventricular hemorrhage (IVH), which afflicts thousands of people of all ages every year, frequently results in the development of communicating hydrocephalus. Classically, IVH-induced hydrocephalus has been attributed to reduced resorption of cerebrospinal fluid (CSF) due to dysfunction of arachnoid granulations, but this explanation may be incomplete. We hypothesized that IVH would cause inflammation of the choroid plexus and of the ependymal lining of the ventricles, resulting in dysfunction of these barrier cells. Barrier dysfunction, in turn, would be expected to cause an increase in production of abnormal protein-rich CSF and transependymal migration of CSF. We tested this hypothesis using a rat model of IVH, in which 160 μl of autologous blood was infused into the lateral ventricle, resulting in a twofold increase in ventricular size 48 h later. In this model, we found significant activation of nuclear factor κB (NF-κB) signaling by the CSF barrier cells of the choroid plexus and ependymal lining. Moreover, these inflammatory changes were associated with abnormal uptake of serum-derived IgG by the barrier cells, a phenomenon closely linked to abnormal permeability of the blood–brain barrier. We conclude that inflammation marked by NF-κB signaling is a prominent feature after IVH and may account for certain pathophysiological sequelae associated with IVH.

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Correspondence to: J. Marc Simard, msimard@smail.umaryland.edu.

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Keywords

Intraventricular hemorrhage; Inflammation; Nuclear factor kappaB; p65; Hydrocephalus; Transependymal migration

Introduction

Intraventricular hemorrhage (IVH) afflicts thousands of people of all ages every year. Common conditions that give rise to IVH include germinal matrix hemorrhage in premature infants, traumatic brain injury in young adults, aneurysmal subarachnoid hemorrhage in middle-aged patients, hypertensive hemorrhagic strokes involving caudate or thalamus in older patients, and amyloid angiopathy in the elderly. It is widely accepted that the extension of hemorrhage into the ventricle in any of these conditions is a poor prognostic indicator and, if severe enough, may result in death [2, 5]. Patients who survive IVH often suffer marked cognitive and neurofunctional impairments.

Intraventricular hemorrhage frequently results in the development of communicating hydrocepahlus. The pathophysiology underlying IVH-induced hydrocephalus is poorly understood. Classically, IVH-induced hydrocephalus has been attributed to dysfunction of the arachnoid granulations that are responsible for returning cerebrospinal fluid (CSF) to the venous system [3], resulting in an excess accumulation of CSF and an increase in intraventricular pressure. However, this simple explanation may be incomplete. It is recognized that intracerebral and subarachnoid hemorrhage cause inflammation in surrounding brain tissues, and that this results in dysfunction of blood–brain-barrier cells, altered barrier permeability, and production of protein-rich vasogenic edema that increases tissue pressure [8]. By analogy, we reasoned that IVH would cause inflammation of the choroid plexus and of the ependymal lining of the ventricles, resulting in dysfunction of these barrier cells and leading to the production of abnormal protein-rich CSF and transependymal migration of CSF. Here, using a rat model of IVH, we tested the hypothesis that IVH would lead to inflammation of the choroid plexus and of the ependymal lining of the ventricle, and that these changes would be associated with altered handling of serum-derived IgG by CSF barrier cells.

Materials and Methods

Surgical procedures were approved by the Institutional Animal Care and Use Committee of the University of Maryland. Adult male rats (Wistar; 300–350 g) were anesthetized (60 mg/kg ketamine plus 7.5 mg/kg xylazine i.p.) and allowed to breathe air spontaneously. Using aseptic techniques, the tail artery was cannulated (PE-20), with patency maintained using heparinized saline. The rat was mounted in a stereotactic apparatus (Stoelting). Core temperature was maintained at 37±1°C using a heating pad. Using aseptic techniques, a midline incision was used to expose the dorsal surface of the skull, and a high speed drill was used to create a 1-mm burr hole 0.5 mm posterior and 1.5 mm to the right side of the bregma. Under stereotactic guidance (coordinates, x=−0.5, y=+1.5, z=+5.5 mm relative to bregma), 160 μl of autologous blood free of anticoagulants that had been freshly collected from the cannulated tail artery was infused aseptically into the right lateral ventricle over the course of 20 min [6]. Animals infused in the same manner but with 160 μl of sterile normal saline were used as controls.

At 48 h after intraventricular infusion, rats were euthanized, exsanguinated, and perfusion-fixed with 10% paraformaldehyde for histology or immunohistochemistry. Paraffin-embedded sections stained with hematoxylin and eosin were used to measure ventricular

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size. Cryosections were used for immunolabeling, with the primary antibodies directed against the NFκB subunit, p65 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) and rat IgG (1:200; FITC-conjugated, Santa Cruz Biotechnology). Species-appropriate fluorescent-labeled secondary antibodies were used, when needed, for visualization. Omission of primary antibody was used as a negative control.

Quantitative immunohistochemistry was carried out as described [8]. All sections were immunolabeled as a single batch. All images were collected using uniform parameters of magnification and exposure. Segmentation analysis was performed by computing a histogram of the pixel intensity for equal regions of interest (ROI) using Nikon NIS-Elements Advanced Research software, with the ROI selected in micrographs taken with ×20 lens (Nikon 90i) containing choroid plexus tissue. For p65, the overall pixel intensity of the ROI outlining the choroid plexus was determined, and the background was subtracted. For IgG, specific labeling was defined as pixels with signal intensity greater than twice that of background, and the area occupied by pixels with specific labeling was used to determine the percent area with specific labeling (% ROI).

Results

Characterization of the Model

We first assessed the adequacy of the model, specifically, whether the stereotactic coordinates and the methods used for intraventricular infusion yielded intraventricular blood without undue tissue trauma. Photographs taken in the cryostat of the frozen blocks at the coronal level encompassing the needle tract showed that the basal ganglia and other periventricular tissues appeared intact, and that the lateral ventricle as well as the third ventricles was filled with blood (Fig. 1a, b).

We also assessed for hydrocephalus. Paraffin-embedded sections stained with hematoxylin and eosin showed that, at 48 h, this model of IVH was associated with ventriculomegaly involving the third and both lateral ventricles (Fig. 1c) [6]. Measurements of the ipsi- and contralateral ventricular areas 48 h after infusion showed a twofold or greater increase in size in rats infused with blood compared to those infused with the same volume of normal saline (Fig. 1c, d).

Inflammation

We used the nuclear factor κB (NF-κB) subunit, p65, as a marker of an inflammatory response. Immunolabeling showed that p65 expression was visibly upregulated in both the choroid plexus and the ependymal layer of rats with IVH compared to control rats with intraventricular saline infusion (Fig. 2a–d). In sections immunolabeled for p65 (red) and stained with 4′,6-diami-dino-2-phenylindole (DAPI) to label nuclei (blue), nuclear translocation of p65 was confirmed by the pink appearance of nuclei in choroid plexus cells (Fig. 2e, arrows). No nuclear p65 was observed in the choroid plexus of any saline-infused rat. Quantitative immunohistochemistry showed a twofold increase in p65 in the choroid plexus with IVH compared to controls (Fig. 2f), indicating a significant upregulation of NF-κB subunit expression.

Immunoglobulin G

Normally, the barrier cells of the CNS show little intracellular uptake of IgG, but when stressed or activated by injury, they can exhibit a significant increase in intracellular uptake of IgG that correlates with altered barrier permeability [9]. Immunolabeling showed that IgG uptake was visibly increased in both the choroid plexus and the ependymal layer of rats with IVH compared to control rats with intraventricular saline injection (Fig. 3a–d). Quantitative

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immunohistochemistry showed a several-fold increase in IgG in the choroid plexus with IVH compared to controls (Fig. 3e), suggesting a significant alteration in barrier permeability.

Discussion

A characteristic feature of an inflammatory response is upregulation and activation of NF-κB signaling. To our knowledge, this is the first report to demonstrate that IVH of a magnitude sufficient to cause hydrocephalus is associated with significant activation of NF-κB signaling by the CSF barrier cells of the choroid plexus and ependymal lining. Our rationale for studying this question originated with the recognition that, in brain parenchyma, extravasated blood causes an inflammatory response in microvascular barrier cells that results in dysfunction of tight junction complexes between endothelial cells [8]. Dysfunction of tight junctions, in turn, gives rise to altered permeability of the blood–brain barrier and formation of protein-rich “vasogenic edema”, resulting in an increase tissue pressure and exposure of brain cells to potentially toxic substances.

We hypothesized that a similar phenomenon might occur after exposure of CSF barrier cells to extravasated blood. In this study, we observed not only nuclear translocation of p65 but also upregulation of p65 expression, which together imply active NF-κB signaling typical of an inflammatory response. Moreover, this cellular response was associated with abnormal cellular uptake of IgG by choroid plexus epithelial cells, paralleling observations of increased uptake of IgG in capillary endothelial cells following injury [9]. In choroid plexus epithelial cells, IgG uptake may represent a reversion to transcellular passage of protein via epithelial cells, similar to that reported in the immature brain [4]. We speculate that these abnormalities could profoundly affect the character of the CSF produced, possibly resulting in excess production of CSF that is abnormally enriched in protein and other constituents of plasma. Such pathophysiological effects of IVH would compound the abnormality in CSF resorption that classically has been held to be solely responsible for IVH-induced hydrocephalus.

We also observed an increase in NF-κB signaling in the ependymal cells lining the ventricle. As with the choroid plexus, this cellular response by ependymal cells was also associated with abnormal cellular uptake of IgG. The functional consequences of excess IgG uptake by ependymal cells have not been determined, but extrapolating from knowledge of this phenomenon in other CNS barrier cells leads to the possibility that this barrier function may have been compromised. It is intriguing to speculate that inflammation of the ependymal lining could give rise to “transependymal migration of CSF”, a radiographically defined phenomenon [7] that is poorly understood in terms of both etiology and functional consequence.

Summary

The choroid plexus is involved in a variety of neurological disorders, including Alzheimer’s and other neurodegenerative diseases, multiple sclerosis and other inflammatory conditions, as well as infectious, traumatic, neoplastic, and systemic diseases [1, 10]. The observations reported here suggest that further study may be warranted of the abnormalities in the barrier cells of the choroid plexus and ependyma that are brought forth by IVH.

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References

1. Engelhardt B, Sorokin L. The blood–brain and the blood–cerebrospinal fluid barriers: function and dysfunction. Semin Immunopathol. 2009; 31:497–511. [PubMed: 19779720]
2. Hanley DF. Intraventricular hemorrhage: severity factor and treatment target in spontaneous intracerebral hemorrhage. Stroke. 2009; 40:1533–8. [PubMed: 19246695]
3. Hudgins RJ. Posthemorrhagic hydrocephalus of infancy. Neurosurg Clin N Am. 2001; 12:743–51. [PubMed: 11524295]
4. Johansson PA, Dziegielewska KM, Liddelow SA, et al. The blood–CSF barrier explained: when development is not immaturity. Bioessays. 2008; 30:237–48. [PubMed: 18293362]
5. Jordan LC, Kleinman JT, Hillis AE. Intracerebral hemorrhage volume predicts poor neurologic outcome in children. Stroke. 2009; 40:1666–71. [PubMed: 19286576]
6. Lodhia KR, Shakui P, Keep RF. Hydrocephalus in a rat model of intraventricular hemorrhage. Acta Neurochir Suppl. 2006; 96:207–11. [PubMed: 16671456]
7. Matsumura K, Matsuda M, Handa J, et al. Magnetic resonance imaging with aneurysmal subarachnoid hemorrhage: comparison with computed tomography scan. Surg Neurol. 1990; 34:71–8. [PubMed: 2367934]
8. Simard JM, Geng Z, Woo SK, et al. Glibenclamide reduces inflammation, vasogenic edema, and caspase-3 activation after subarachnoid hemorrhage. J Cereb Blood Flow Metab. 2009; 29:317–30. [PubMed: 18854840]
9. Simard JM, Yurovsky V, Tsymbalyuk N, et al. Protective effect of delayed treatment with low-dose glibenclamide in three models of ischemic stroke. Stroke. 2009; 40:604–9. [PubMed: 19023097]
10. Wolburg H, Paulus W. Choroid plexus: biology and pathology. Acta Neuropathol. 2010; 119:75–88. [PubMed: 20033190]

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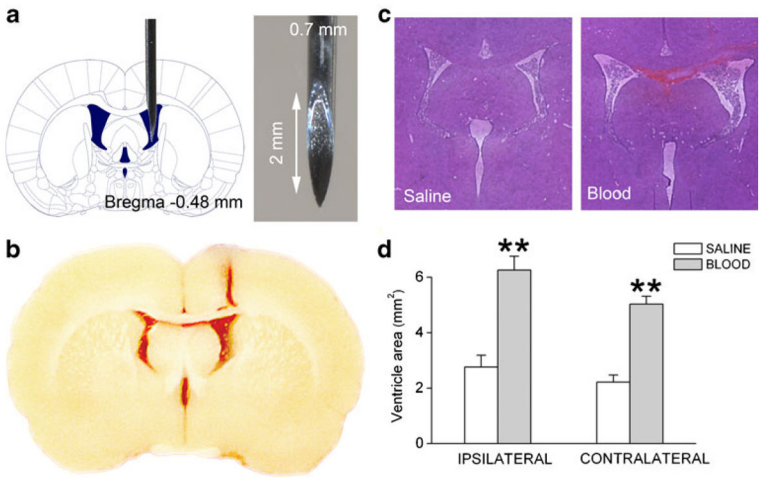

Fig. 1.

The model of intraventricular hemorrhage. **a** Stereotactic technique used to produce IVH in the rat; the size of the infusion needle and the ventricle are shown to scale. **b** Intraventricular hemorrhage involving the lateral and third ventricles; the photograph was taken from within the cryostat of the frozen block of brain at the location of needle placement. **c** Images of paraffin sections stained with hematoxylin and eosin, showing the ventricles 48 h after infusion of 160 μl of normal saline or of autologous blood. **d** Measurements of the area of the ipsilateral and contralateral ventricles at 48 h; five rats per group; $^{**}P<0.01$

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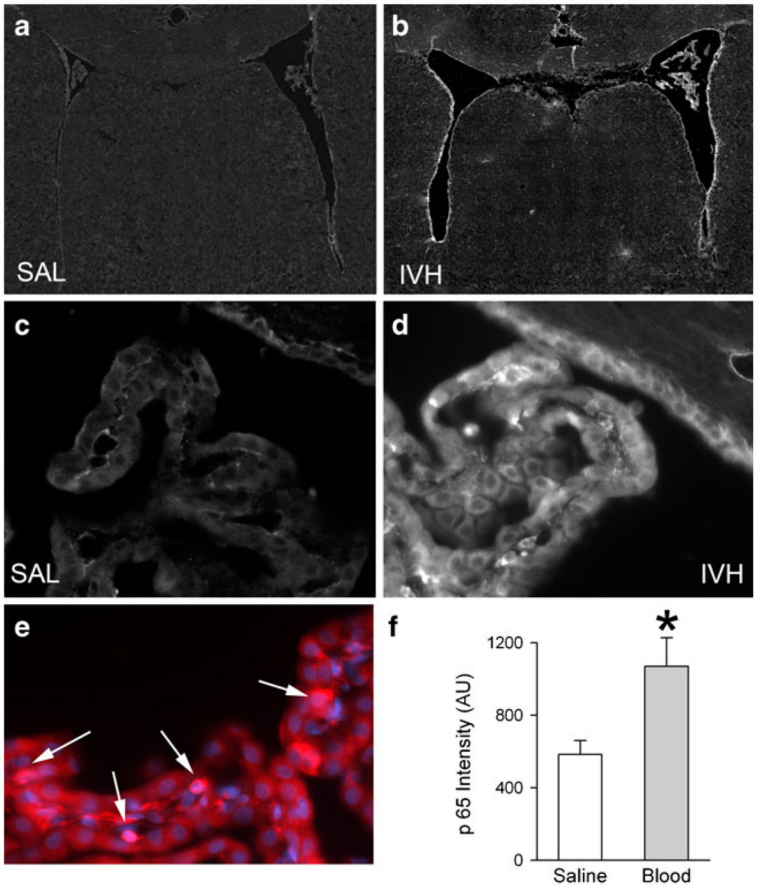

Fig. 2.

Intraventricular hemorrhage causes activation of NF-κB. **a**–**e** Low magnification (**a, b**) and high magnification (**c**–**e**) views of coronal sections immunolabeled for p65 48 h after infusion of saline (*SAL*) or blood (*IVH*), as indicated; the section in (**e**) was also stained with DAPI to show nuclei (*blue*), with *pink nuclei* indicating nuclear translocation of p65 (*arrows*). **f** Quantitative immunohistochemistry for p65 showed significantly greater expression following exposure to blood; five rats per group; **P*<0.05

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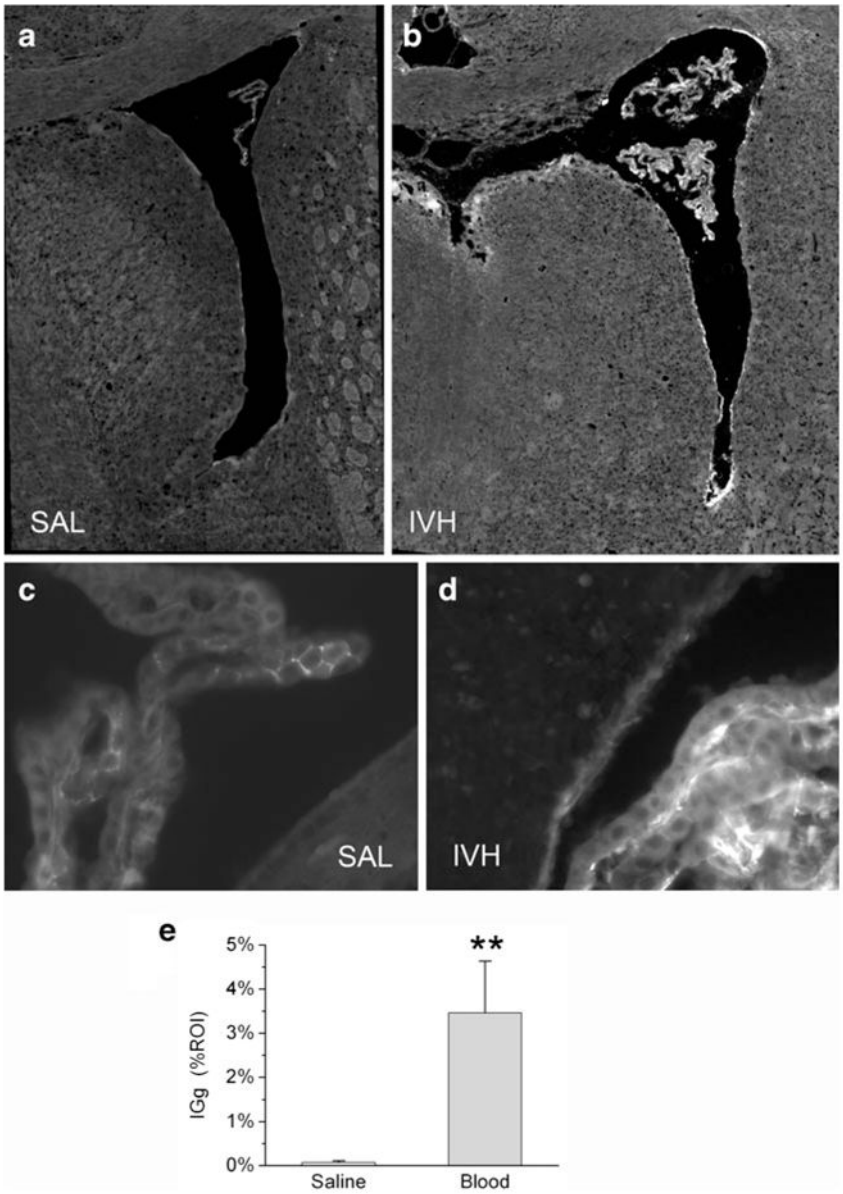

Fig. 3.

Intraventricular hemorrhage causes abnormal uptake of immunoglobulin G by barrier cells. **a**–**e** Low magnification (**a**, **b**) and high magnification (**c**, **d**) views of coronal sections immunolabeled for IgG 48 h after infusion of saline (*SAL*) or blood (*IVH*), as indicated. **f** Quantitative immunohistochemistry for IgG showed significantly greater uptake following exposure to blood; five rats per group; ***P*<0.01