Photochemically Stimulated Blood-Borne Factors Induce Blood–Brain Barrier Alterations in Rats

W. Dalton Dietrich, PhD, Ricardo Prado, MD, and Brant D. Watson, PhD

We have tested the hypothesis that blood-borne substances released from a site of vascular thrombosis can lead to acute alterations in the blood–brain barrier. The right common carotid artery of rats was photothrombosed using a dye/light insult. Rats were given the photosensitizing dye rose bengal and irradiated for 4 minutes with an argon laser beam focused onto the exposed common carotid artery. During the irradiation, 3 ml of blood was taken from the right external carotid artery. After 10 minutes, the blood was infused into the external carotid artery of a recipient rat that had received horseradish peroxidase. Fifteen minutes after blood infusion, bilateral peroxidase extravasation was noted within cortical and subcortical areas of recipient rats, being most intense ipsilaterally. Ultrastructural studies demonstrated peroxidase reaction product within numerous endothelial vesicles of arteriolar segments. Infusion of blood from control rats did not produce similar changes. Thus, photoinduced vascular thrombosis of a large feeding artery leads to the formation of blood-borne factors that acutely alter cerebral vascular permeability. (Stroke 1988;19:857-862)

Abnormalities of blood rheology are believed to play an important role in the pathophysiology of stroke. Altered platelet function, increased hematocrit, and decreased erythrocyte deformability are just a few of the changes that have been detected in patients with neurologic deficits secondary to cerebral ischemia. Blood constituents have also been implicated in postischemic reperfusion abnormalities. In one experimental investigation, the preischemic modification of an animal’s blood by exposure to glass wool greatly enhanced postischemic cerebral reperfusion. Studies such as these demonstrate that cerebral ischemia can produce blood alterations that in some cases may be detrimental to postischemic brain recovery. However, whether blood abnormalities also represent a primary event in the occurrence of acute stroke still remains unclear. For example, it is not known whether vascular injury in the absence of cerebral ischemia also leads to the formation of blood-borne factors that acutely affect brain structure and function.

Experimentally induced thrombosis of neocortical vessels can be produced in rats by means of a rose bengal dye-sensitized photochemical reaction facilitated by irradiation with a filtered xenon arc lamp. This insult leads to acute alterations in the blood–brain barrier (BBB) and to vasogenic edema within the cortical region destined to undergo ischemic infarction. Recently, vascular endothelial damage leading to thrombosis of the middle cerebral artery (MCA) and common carotid artery (CCA) has been accomplished using the focused beam of an argon laser as the light source. Photothrombosis of the MCA also resulted in rapid alterations in cerebral vascular permeability, with bilateral leakage of horseradish peroxidase (HRP) 15 minutes after thrombus formation. Because protein extravasation was present in the contralateral hemisphere, it was hypothesized that mechanisms responsible for extravasation include blood-borne substances released at the site of primary thrombosis.

To directly test this hypothesis, we carried out a series of studies to determine whether blood from a rat undergoing arterial thrombosis would alter vascular permeability in a recipient rat. To this end, rats underwent unilateral CCA photothrombosis and blood was collected from the ipsilateral external carotid artery (ECA). Ten minutes later the blood was infused into the ECA of a recipient rat, and the integrity of the BBB was assessed with HRP.
FIGURE 1. Scanning electron micrographs of irradiated common carotid artery from donor rat. a: Platelet thrombi are associated with damaged endothelial surface. Bar=10 μm. b: Higher magnification of damaged endothelial cells and adhering platelets. Bar=10 μm.

Materials and Methods

We performed the experiments on 20 heparinized male Wistar rats weighing between 250 and 300 g. In rats destined to undergo CCA thrombosis (donor group, \( n = 6 \)), anesthesia was induced with 3% halothane for 3-5 minutes. Donor rats were then maintained on 1.5% halothane and a mixture of 70% nitrous oxide-30% oxygen delivered by a closely fitting face mask. Femoral artery and venous catheters were inserted for the measurement of arterial blood pressure and blood gases and for fluid administration. The right CCA of a donor rat was exposed using an operating microscope. During cannulation of the right ECA, the CCA was temporarily ligated to inhibit excessive bleeding. After insertion of the cannula, which took 1-2 minutes, the CCA ligature was removed. Rats destined to receive blood (recipient group, \( n = 6 \)) were anesthetized with 3% halothane and paralyzed with 5 mg/kg \( d \)-tubocurarine chloride. Recipient rats underwent tracheostomy and were mechanically ventilated and maintained on 70% nitrous oxide-30% oxygen. The right ECA was isolated and cannulated as above.

Next a donor rat was placed on its back, and the neck region containing the exposed CCA was aligned with the beam of a tunable argon laser (Lexel Model 95, Cooper Lasersonics, Fremont, California) operated at 514.5 nm and a peak power of 1.5 W. The photosensitizing dye rose bengal, 40 mg/kg in 0.9% saline, was injected over 1 minute simultaneous with the start of irradiation. The irradiating beam was focused through a 61-cm-focal-length spherical lens onto the CCA as a spot enveloping the diameter of the artery. The irradiating beam was interrupted by a mechanical beam chopper operated at 200 Hz and a duty cycle of 20%. The CCA was irradiated for 4 minutes, during which time 3 ml of blood was removed from the right ECA and collected in a heparinized syringe. A recipient rat was then injected intravenously over 2 minutes with 30 mg/ml HRP (Type 2, Sigma Chemical Company, St. Louis, Missouri) dissolved in 1 ml saline. Blood pressure and arterial blood gases were monitored throughout the experiment. Ten minutes later, blood from a donor rat was infused slowly over 2 minutes into the ECA of a recipient rat while a similar volume of blood was removed from the femoral artery.

In control studies, rats underwent all donor experimental procedures except they were either irradiated but not given rose bengal or were given rose bengal and not irradiated. Blood from control rats was infused into control-recipient rats (\( n = 3 \)) as above.

Fifteen minutes after the end of blood infusion, donor, recipient, and control-recipient rats were perfused transcardially with 0.9% saline followed by 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer. Perfusion pressure was maintained at 100 mm Hg. After perfusion fixation, irradiated segments of the donors' CCA and brains of the recipient rats were placed in chilled fixative (4° C) for 1 hour. CCA segments and brains were then transferred to chilled 0.1 M sodium phosphate buffer (4° C) for 2 hours. CCA segments were cut longitudinally and processed for scanning electron microscopy. The brains were blocked into coronal segments and mounted on a Vibratome and sectioned at 50 or 100 μm in the coronal plane. Sections were then reacted with 3,3'-diaminobenzidine for the demonstration of HRP. Light microscopic analysis was carried out on 100-μm Vibratome sec-
Dietrich et al  
BBB Alterations After Arterial Thrombosis

FIGURE 2. Patterns of horseradish peroxidase (HRP) leakage in cleared Vibratome sections.  
a: Section from rat infused with blood from control rat.  Note relative absence of HRP leakage.  
b: Bilateral HRP extravasation is prominent in section from rat infused with blood from rat subjected to common carotid artery thrombosis.  
c: Within ipsilateral cortical regions of rat in b, HRP leakage is associated with large vessels coursing perpendicular to pial surface.  
Focal areas of HRP leakage are also present within striatum.  
d: Neuronal cell bodies and cell processes flooded with HRP are 
associated with leaky cortical arteriole in section from rat infused with blood from rat subjected to common carotid artery thrombosis.

Physiologic and Control Findings

The physiologic findings of the recipient group were within normal ranges:  
\( P_{CO_2} \), 42.8 ± 4.2 mm Hg (mean ± SD);  
\( P_{O_2} \), 119.4 ± 13.0 mm Hg;  
pH, 7.40 ± 0.03, mean arterial blood pressure, 120.5 ± 10.5 mm Hg.  
Hypotension or hypertension were not recorded during or after the blood infusion.  

Widespread permeability alterations were not demonstrated in control-recipient rats.  
In one, a leaky arteriole was detected within the ipsilateral striatum, whereas in a second, HRP extravasation was confined to cortical and striatal vascular segments ipsilateral to the cannulated ECA (Figure 2a).  
HRP leakage was not detected in the contralateral hemisphere.

Experimental Findings

Islets of platelet aggregates associated with a damaged endothelial surface were detected by scanning electron microscopy within the irradiated CCA of donor rats (Figure 1).  
Complete occlusion of the lumen by platelet thrombi was not seen.  
Individual platelets with pseudopodia were shown adhering to damaged endothelium and exposed subendothelial layers (Figure 1b).

Whole blood from donor rats engendered widespread alterations in HRP extravasation within cortical and subcortical brain regions of recipient rats (Figure 2, b and c).  
Within ipsilateral neocortical regions, leakage involved frontal, frontoparietal somatosensory, and motor cortices.  
At these sites HRP leakage was patchy and frequently associated with large penetrating vessels (Figure 2c).  
Subcortically, focal areas of HRP extravasation were consistently detected within the ipsilateral striatum and within basal forebrain regions.  
Extravasated HRP was located within vascular walls, pericytes, and surrounding brain parenchyma.  
Within cortical

Sections that had been mounted and air-dried on glass slides, dehydrated, cleared, and coverslipped.  
For transmission electron microscopic analysis, areas of interest were dissected from 50-\( \mu \)m sections immersed in buffer and processed as described.  

Results

Physiologic and Control Findings

The physiologic findings of the recipient group were within normal ranges:  
\( P_{CO_2} \), 42.8 ± 4.2 mm Hg (mean ± SD);  
\( P_{O_2} \), 119.4 ± 13.0 mm Hg;  
pH, 7.40 ± 0.03, mean arterial blood pressure, 120.5 ± 10.5 mm Hg.  
Hypotension or hypertension were not recorded during or after the blood infusion.  

Widespread permeability alterations were not demonstrated in control-recipient rats.  
In one, a leaky arteriole was detected within the ipsilateral striatum, whereas in a second, HRP extravasation was confined to cortical and striatal vascular segments ipsilateral to the cannulated ECA (Figure 2a).  
HRP leakage was not detected in the contralateral hemisphere.

Experimental Findings

Islets of platelet aggregates associated with a damaged endothelial surface were detected by scanning electron microscopy within the irradiated CCA of donor rats (Figure 1).  
Complete occlusion of the lumen by platelet thrombi was not seen.  
Individual platelets with pseudopodia were shown adhering to damaged endothelium and exposed subendothelial layers (Figure 1b).

Whole blood from donor rats engendered widespread alterations in HRP extravasation within cortical and subcortical brain regions of recipient rats (Figure 2, b and c).  
Within ipsilateral neocortical regions, leakage involved frontal, frontoparietal somatosensory, and motor cortices.  
At these sites HRP leakage was patchy and frequently associated with large penetrating vessels (Figure 2c).  
Subcortically, focal areas of HRP extravasation were consistently detected within the ipsilateral striatum and within basal forebrain regions.  
Extravasated HRP was located within vascular walls, pericytes, and surrounding brain parenchyma.  
Within cortical
regions showing focal areas of extravasation, HRP flooded neuronal cell bodies and processes (Figure 2d). Leaky vessels were also detected bordering on or coursing within the corpus callosum and anterior commissure.

The degree of HRP leakage decreased as more posterior brain sections were analyzed. At the level of the hippocampus and thalamus, diffuse areas of HRP extravasation were encountered. The internal capsule demonstrated focal HRP leakage in two of six recipient rats.

Alterations in vascular permeability were also documented contralateral to the injected ECA (Figure 2b). These alterations in vascular permeability were less dramatic than those seen ipsilaterally and consisted of a more diffuse pattern. Within cortical regions, HRP leakage was restricted to deeper cortical layers. Diffuse leakage was also detected within the dorsolateral striatum, hippocampus, and thalamus.

Ultrastructural analysis of leaky areas demonstrated arteriolar segments with extravasated HRP (Figure 3). Endothelial nuclei sometimes appeared slightly constricted, and endothelial microvilli were detected extending into the vessel lumen (Figure 3a). Pinocytotic vesicles containing HRP were associated with luminal and abluminal endothelial surfaces (Figure 3b). Various degrees of HRP flooding into surrounding basal lamina and perivascular spaces were documented in leaky areas. Tight junctions appeared intact, and severe damage to the endothelial lining or muscular layers was not seen. Luminal blood elements were not consistently detected within leaky vessels. Occasional HRP-containing pinocytotic vesicles were seen within the endothelium of capillaries and venules.

Discussion

Our results demonstrate that whole blood collected downstream from a thrombosed artery can alter the vascular permeability of the brain. In our study, thrombosis of the CCA was produced by a photochemical reaction, which has been discussed.11,13 Endothelium-bound rose bengal molecules are excited by the laser light, resulting in the production of singlet molecular oxygen, which may then initiate direct peroxidation of unsaturated fatty acids and proteins of the irradiated endothelial cells. The ultrastructural characteristics of photochemically induced endothelial injury and subsequent platelet activation have also been described.11,16,17 Because the photochemical insult produces both endothelial injury and the possible activation of several classes of blood cells, the mechanism(s) responsible for the permeability effects are likely to be complex.

Cerebral microembolization acutely alters the BBB.18 A few leaky vessels were detected in two of three control-recipient rats. It is possible that microemboli formed during cannulation contributed to these BBB alterations. In the experimental series, platelet emboli produced by the photochemical insult in the donor rats may have been collected in the heparinized ECA cannula and infused into the recipient rats. Although we did not obtain direct evidence for mechanical occlusion of the leaky microvascular beds by platelet emboli, vasoactive substances released by activated platelets may be involved in the permeability changes. Hormonal control of the BBB has been discussed, and platelets as well as endothelial cells and leukocytes synthesize factors that influence vascular permeability.19-21 For exam-
ple, serotonin is released from aggregating platelets and alters the permeability characteristics of pial venules by a receptor-mediated process.

Leukocytes normally interact with vascular endothelium and have been shown to influence BBB permeability in several microvascular beds. As with platelets, leukocytes were not consistently observed in areas of HRP extravasation, and therefore, microvascular plugging by leukocytes may not be responsible for the permeability changes. However, it is possible that under our experimental conditions, leukocytes were activated as they passed through the damaged CCA segment by chemical products of endothelial injury or platelet aggregation, thereby contributing to the remote BBB permeability alterations. In this regard, leukocyte activation leading to enhanced leukocyte–endothelial interactions and BBB alterations has been suggested to occur in some immunopathologic conditions.

Ultrastructural analysis of arteriole segments showed that the HRP was extravasated via transendothelial pinocytotic transport and not by disruption of endothelial membranes or alterations in tight junctions. These BBB changes may be reversible, with normal function returning once the bloodborne stimulus is inactivated or removed from the blood. Severe edema resulting in brain swelling and secondary microvascular compression is not likely to occur under our experimental conditions. Astrocytes swell as edema develops, and only mild perivascular swelling was detected around leaky vessels. Nevertheless, these permeability changes could allow neuroactive substances to cross the BBB and produce either transient or more permanent parenchymal consequences.

Neuronal flooding with various protein tracers following BBB disruption has been documented in several experimental conditions including cerebral ischemia and mechanical brain injury. Some studies have suggested that neuronal flooding signals irreversible neuronal damage whereas others have demonstrated that flooded neurons may be perturbed only transiently and are not necessarily destined to die. Because our recipient rats were allowed to survive for only 15 minutes after blood infusion, the fate of the flooded neurons remains uncertain. Nevertheless, the fact that neuronal alterations were documented suggests that acute neuronal dysfunction may be a direct consequence of altered vascular permeability.

In summary, experimentally induced thrombosis of a large feeding artery produces blood-borne substances that acutely affect vascular permeability to proteins throughout the brain. Whether bloodborne factors induce similar BBB alterations in patients during a thrombotic event is an obvious but unanswered question. The BBB may be necessary for “higher” functions of neural tissue. It therefore seems justified that the functional consequences of acute vascular thrombosis be explored experimentally.

Acknowledgments

We wish to thank our colleagues Drs. Myron Ginsberg and Peritz Scheinberg for helpful comments, Marcilia Halley for technical assistance, and Helen Valkovitz for typing.

References

21. Olesen S-P, Crane C: Substances that rapidly augment ionic transport and not by disruption of endothelial membranes or alterations in tight junctions. These BBB changes may be reversible, with normal function returning once the bloodborne stimulus is inactivated or removed from the blood. Severe edema resulting in brain swelling and secondary microvascular compression is not likely to occur under our experimental conditions. Astrocytes swell as edema develops, and only mild perivascular swelling was detected around leaky vessels. Nevertheless, these permeability changes could allow neuroactive substances to cross the BBB and produce either transient or more permanent parenchymal consequences.

Neuronal flooding with various protein tracers following BBB disruption has been documented in several experimental conditions including cerebral ischemia and mechanical brain injury. Some studies have suggested that neuronal flooding signals irreversible neuronal damage whereas others have demonstrated that flooded neurons may be perturbed only transiently and are not necessarily destined to die. Because our recipient rats were allowed to survive for only 15 minutes after blood infusion, the fate of the flooded neurons remains uncertain. Nevertheless, the fact that neuronal alterations were documented suggests that acute neuronal dysfunction may be a direct consequence of altered vascular permeability.

In summary, experimentally induced thrombosis of a large feeding artery produces blood-borne substances that acutely affect vascular permeability to proteins throughout the brain. Whether bloodborne factors induce similar BBB alterations in patients during a thrombotic event is an obvious but unanswered question. The BBB may be necessary for “higher” functions of neural tissue. It therefore seems justified that the functional consequences of acute vascular thrombosis be explored experimentally.
increase in microvessels in frog brain. J Physiol 1985; 361:103-113

KEY WORDS • blood–brain barrier • permeability • thrombosis • rats
Photochemically stimulated blood-borne factors induce blood-brain barrier alterations in rats.
W D Dietrich, R Prado and B D Watson

Stroke. 1988;19:857-862
doi: 10.1161/01.STR.19.7.857

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/19/7/857

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/