Section Editors: Anna M. Planas, PhD, and Midori A. Yenari, MD

Brain Regulation of Thrombosis and Hemostasis
From Theory to Practice

Mark J. Fisher, MD

Thrombosis and hemostasis impact stroke neurology and cerebrovascular disease. Decision making for stroke prevention and stroke treatment almost invariably involves pharmacotherapies that involve coagulation, with drugs ranging from aspirin to anticoagulants, both new and old. Nevertheless, the focus on pharmacotherapy modulation of coagulation processes tends to overlook some basic pathophysiological realities: the organ affected by stroke has its own unique system for regulation of thrombosis and hemostasis.

The purpose of this article is to review the brain's intrinsic capacity for thrombosis and hemostasis regulation. At first glance, it may seem that this is esoteric subject matter. Indeed, until recently, it seemed that this material was largely of theoretical interest.

It is now evident that thrombosis, hemostasis, and the brain have enormous practical significance for the field of stroke neurology. Intrinsic regulation of thrombosis and hemostasis can no longer be ignored if one is to intervene pharmacologically in ways that impact coagulation. Manipulation of systemic coagulation factors will necessarily have consequences implicating intrinsic brain regulatory mechanisms.

This article will review thrombosis and hemostasis from an organ-specific perspective and one organ in particular: the brain. The article will address how brain regulation of thrombosis and hemostasis manifests itself in the context of organ-specific regulation. The phenomenon of cerebral microbleeds will then be discussed as a pivotal and perhaps paradigmatic example of the importance of this issue.

Thrombosis, Hemostasis, and Coagulation

The terms thrombosis, hemostasis, and coagulation are sometimes used interchangeably, but more precisely refer to different processes. Hemostasis defines the avoidance or arrest of bleeding by maintaining blood within a vessel. Thrombosis is the formation of clot within a blood vessel, resulting in obstruction of flow, whereas coagulation refers to a liquid transformed into a coherent solid or semisolid mass.[1]

Coagulation is primarily regulated by circulating soluble factors, circulating cells, and vessel wall constituents, combined with vascular integrity and blood flow. Circulating cells are considered the components of primary hemostasis, whereas circulating coagulation factors constitute secondary hemostasis.[1] The 4-component model of coagulation (hepatic factors, bone marrow–derived hematopoietic cells, vascular tree, and endothelium) represents a useful conceptual approach to this complex system, in which primary hemostasis consists of the relevant bone marrow–derived cells (platelets and monocytes) while the liver provides the coagulation factors of secondary hemostasis.[1]

Another model incorporates the classical Virchow triad, consisting of alterations of blood flow, blood wall, and blood constituents. This model is typically used to explain predilection for thrombosis but is also highly relevant for maintenance of hemostasis.[1–3] Although flow reduction, overproduction of coagulation factors, and exposure of subendothelial constituents all contribute to thrombosis, low levels of coagulation factors and disruption of vascular integrity will obviously impact hemostasis.

The process of coagulation (described in some detail in Figure 1) is initiated by activation of factor VII by tissue factor, and this extrinsic pathway activation is amplified by the intrinsic pathway.[4] Coagulation activation leads to fibrin clot generation, and this process is largely regulated by 4 pathways, 3 of which are endothelial-based: the thrombomodulin-protein C pathway, the tissue factor pathway inhibitor (TFPI) pathway, and the fibrinolytic pathway. Another important pathway involves circulating antithrombin III and cofactor heparan sulfate proteoglycans (HSPG), which inhibit all coagulation proteases of the coagulation cascade.[4,5] Studies on knockout mice demonstrate that all 3 anticoagulant pathways are necessary for coagulation cascade regulation; in contrast, animals lacking components of fibrinolytic pathway generally survive until adulthood, suggesting overlap of function for tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA).[4]

Organ-Specific Thrombosis and Hemostasis

Based on Virchow triad, it would be expected that substantial alteration of circulating coagulation factors could result in diffuse thrombotic or hemorrhagic phenomena. And yet this is not the case, because focal or mutlifocal rather than diffuse events occur within the vasculature with these

Received April 24, 2013; final revision received July 12, 2013; accepted July 25, 2013.

From the Departments of Neurology, Anatomy & Neurobiology, and Pathology & Laboratory Medicine, UC Irvine School of Medicine, Irvine, CA.
Correspondence to Mark J. Fisher, MD, Department of Neurology, UC Irvine Medical Center, 101 The City Drive South, Shanbrom Hall, Room 121, Orange, CA 92868. E-mail mfisher@uci.edu

(*Stroke*. 2013;44:3275-3285.)

***Stroke* is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.113.000736**

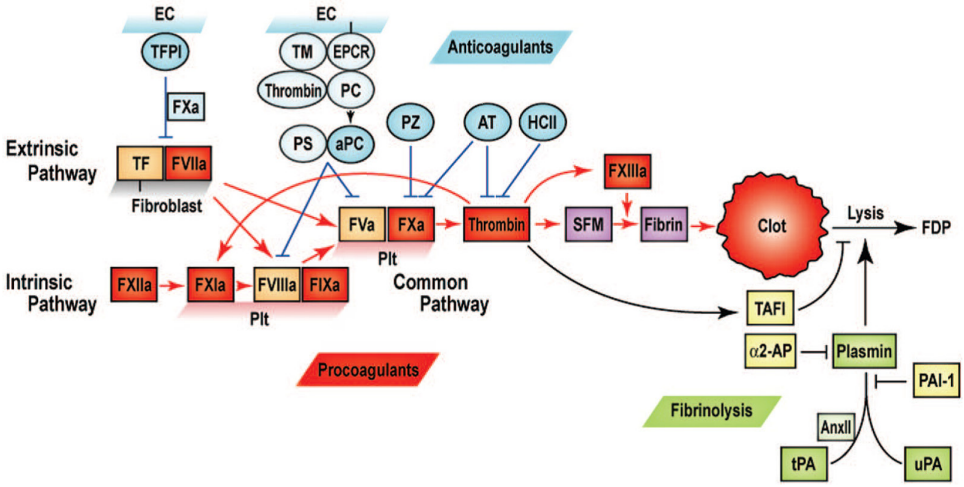

Figure 1. The complexities of the coagulation pathways are illustrated here. The effects of tissue factor (TF)–mediated activation of factor VII (FVIIa), representing extrinsic pathway activation, are amplified by the intrinsic pathway; this results in thrombin activation and generation of soluble fibrin monomer (SFM) and fibrin clot. This procoagulant pathway is negatively regulated by 3 anticoagulant pathways, 2 of which are derived from endothelial cells (EC): tissue factor pathway inhibitor (TFPI), which forms a quaternary complex with TF, FVIIa, and factor Xa (FXa); and the thrombomodulin (TM)–protein C (PC) pathway, in which the TM–thrombin complex activates PC, a process which is amplified by the endothelial protein C receptor (EPCR) with the resulting activated protein C (aPC) and its cofactor protein S (PS) then capable of inactivating factor Va (FVa) and factor VIIIa (FVIIIa). The third major anticoagulant pathway consists of circulating antithrombin III (AT), which can inhibit thrombin, FXa, and other serine proteases; actions of AT are vastly amplified by its binding with endothelial-derived heparan sulfate proteoglycans (not shown). FXa is also inhibited by protein Z (PZ), whereas heparin cofactor (HC II) is another thrombin inhibitor. Fibrin dissolution is produced by the fibrinolytic pathway, with plasmin-induced clot lysis producing fibrin degradation products (FDP). Plasmin is derived from tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) actions on plasminogen. Both tPA and uPA are inhibited by plasminogen activator inhibitor-1 (PAI-1), whereas tPA effects are amplified by annexin II (AnxII). Other negative regulators of fibrinolysis include α2-antiplasmin (α2-AP) and thrombin-activatable fibrinolysis inhibitor (TAFI). Thrombogenic surfaces for the FVa:FXa (prothrombinase) complex, the FVIIIa:factor IXa (FIXa) (intrinsic tenase) complex, and for factor XIa (FXIa) are provided by platelets (Plt). Factor XIIa (FXIIa) may participate in initiation and propagation of clot, whereas factor XIIIa stabilizes thrombus by crosslinking fibrin monomers. Reprinted from Mackman[4] with permission of the publisher.

changes in circulating factors. These local events, occurring in the presence of systemic changes of the coagulation system, are indicative of organ-specific regulation of thrombosis and hemostasis.[5,6]

Focal changes in the presence of a systemic prothrombotic state are evident in diseases of veins, arteries, and microvessels. There seems to be a dual basis for this focality, residing in the differential expression of anticoagulant and procoagulant factors within different elements of the vascular tree, along with specific effects of different organs.[6] For example, the endothelial protein C receptor (EPCR) is expressed predominantly in large arteries and veins, whereas TFPI is principally in capillaries.[6,7] Other endothelial-dependent anticoagulant (eg, nitric oxide) and procoagulant (von Willebrand factor) molecules show predilection for arteries and veins, respectively.[6,8]

Arterial thromboses are particularly dependent on loss of vascular integrity with consequent exposure of subendothelial surfaces to blood.[6] Nevertheless, thrombotic occlusion of the coronary arteries does not substantially increase with deficiencies of the protein C, protein S, or antithrombin III pathway.[5] On the contrary, deficiencies of these same factors clearly predispose to venous thrombosis.[5,6] These venous thromboses tend to occur in the lower extremities, at sites of venous valve pockets where stagnation of flow and local hypoxia are common.[6,9] A vastly different distribution of thrombosis occurs in the presence of polycythemia vera, paroxysmal nocturnal hemoglobinuria, and essential thrombocythemia, in which intraabdominal veins (where valves are sparse, if present at all) are common sites of thrombi.[5,10]

Further demonstration of the complexity of thrombus distribution is observed in the smaller vessels. The syndrome of erythromylagia, due to thrombotic occlusion of arterioles, tends to localize to the toes and fingers and is provoked by presence of myeloproliferative syndromes polycythemia vera and essential thrombocythemia.[6,11] In contrast, warfarin-induced skin necrosis tends to occur in buttocks, thighs, and breasts and is due to thrombotic occlusion of dermal venules in the presence of low circulating protein C.[5,6,12]

The concept of organ-specific regulation has been more frequently applied to thrombosis rather than hemostasis. Nevertheless, there are notable differences in the distribution of hemorrhage, with variations observed in both murine models and humans.[4] The principal differences seem to depend on whether there are deficiencies of the extrinsic or intrinsic pathways. For example, low expression of tissue factor and low levels of factor VII both lead to murine hemorrhage in heart, lung, testis, uterus, and placenta.[4] Deficiencies of intrinsic pathway, specifically factors VIII or IX, lead to hemorrhage involving muscles and joints, where tissue factor expression is known to be low.[4]

Additional evidence for organ-specific regulation of thrombosis derives from a variety of knockout mice, involving tPA, thrombomodulin, and TFPI.[13–18] Initial reports of mice with combined deficiency of both tPA and uPA, with or without endotoxin injection to provoke thrombosis, showed extensive

fibrin deposition in liver, lung, intestine, and uterus; thrombosis of brain was not reported.[13] A later study investigating mice deficient for either tPA or uPA demonstrated 10- to 20-fold increased fibrin deposition in vasculature of lung, spleen, heart, and liver. However, brain and kidney were spared thrombosis.[14]

Further studies examined effects of murine thrombomodulin deficiency. Mice with inactivation of both alleles of the thrombomodulin gene demonstrated extensive fibrin deposition in lung; brain was not specifically examined in this study.[15] Studies of mice with heterozygous thrombomodulin deficiency and with modified thrombomodulin containing a single amino acid substitution (producing vastly reduced ability to activate protein C) showed extensive fibrin deposition in lung, heart, spleen, liver. Once again, brain and kidney did not demonstrate this fibrin deposition.[14] The effect of the murine thrombomodulin gene mutation (single amino acid substitution) was further studied using endotoxin, which provoked thrombosis in kidney, heart, spleen, and lung; however, the brain was again spared.[16]

These findings of organ-specific thrombosis (and organ-sparing of thrombosis) were further explored by examining the effects of combining deficiencies of thrombomodulin with tPA/uPA and TFPI deficiency. Studies of single- and double-knockouts for murine thrombomodulin, tPA, and uPA showed that tPA had greatest impact on fibrin deposition in the heart.[17] Tissue deposition of fibrin was also studied in mice heterozygous for TFPI deficiency combined with thrombomodulin mutation (single amino acid substitution), with fibrin demonstrated in liver but not lung or heart. The impact of these combined defects included some evidence of fibrin deposition in pial vasculature.[18]

These murine investigations are consistent with clinical studies indicating the focal and organ-specific nature of thrombosis and hemostasis. The murine investigations also emphasize a unique role for the brain in this organ-specific regulation. The concept of brain-specific regulation is supported by the paucity of brain thrombosis in these various knockout models. Taken together, these findings suggest that the brain, compared with other organs, has less reliance on antithrombotic and fibrinolytic pathways and imply that protection against hemorrhage is a higher priority for the brain.

Characteristics of Brain-Specific Regulation

The central nervous system fits well within the context of organ-specific regulation of thrombosis and hemostasis. The brain microvasculature demonstrates a remarkably consistent pattern of structural and functional organization that offers an unusual degree of protection against hemorrhage. These findings are localized principally at the microvascular endothelial junctions, combined with constituent underexpression of a variety of antithrombotic molecules.

Structural Characteristics

Brain capillaries are well known for their characteristic tight junction features that largely constitute the blood–brain barrier (BBB; Figure 2).[19] This barrier is typically viewed from the perspective of limiting molecular transit into the brain. It is less well appreciated that this same barrier offers substantial protection against hemorrhagic phenomenon.

In systemic capillaries, ladder-like adherens junctions offer the principal structural protection against hemorrhage at the endothelial junction.[20] These junctions are extensively enhanced in brain capillaries by the BBB. The tight junction constituents claudins and occludins provide protection against hemorrhage that goes well beyond adherens junctions.[19] This additional barrier is credited for the relative sparing of the brain in systemic hemorrhagic phenomena, such as thrombocytopenia.[20,21]

Structural protection against hemorrhage in the microvasculature is not limited to tight junctions alone. The BBB pericyte is preferentially localized opposite tight junctions. This characteristic pericyte localization allows for paracrine production of a variety of trophic factors that enhance the BBB.[22] Adjacent astrocytes (Figure 3) were initially described as the principal initiator of paracrine regulation of the BBB,[23] and the relative impact of these two cell types (pericytes and astrocytes) on barrier characteristics varies under different physiological and pathological conditions.[22,24]

Although these paracrine effects are well known, the pericyte offers additional hemorrhagic protection in the form of a structural barrier to crossing the junction, as well as phagocytic function that may further enhance this barrier protection effect.[22] The location of the pericyte opposite the interendothelial junction in effect constitutes a gate, preventing egress of blood constituents (Figure 3). This gatekeeper element of the pericyte, preventing exit of erythrocytes, may then be amplified by the erythrophagocytic function that is well described in systemic pericytes.[25,26]

Pathological and experimental evidence support this role for brain pericytes. A neuropathological study of cerebral microscopic hemorrhage, by electron microscopy, demonstrated iron deposition in a pericyte immediately opposite a tight junction of a brain capillary, consistent with phagocytosis by pericytes of erythrocytes exiting capillaries.[27] Moreover, a mouse knockout model of the pericyte ligand platelet–derived growth factor receptor β, which results in absence of brain microvascular pericytes—with consequent

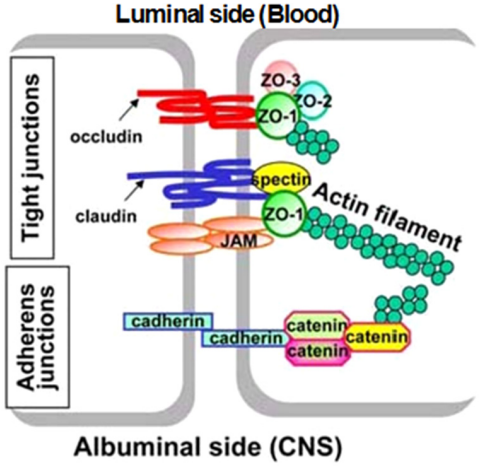

Figure 2. Endothelial tight junctions, with transmembrane molecules occludin and claudin interacting with actin cytoskeleton and zonula occludens (ZO) proteins, including ZO-1. Reprinted from Kim et al[19] with permission of the publisher. Copyright ©2006, BMB Reports (http://www.bmbReports.org/).

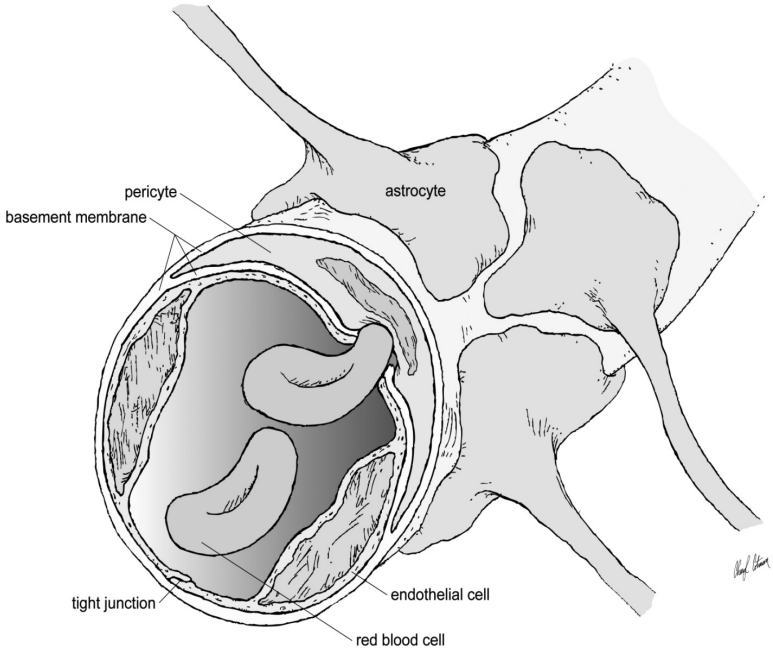

Figure 3. A model illustrating elements of brain-specific hemostasis regulation. A pericyte, opposite capillary tight junction, prevents red blood cell exit, whereas tissue factor–expressing astrocytes provide additional protection against hemorrhage.

BBB dysfunction, microglial activation, and irreversible neuronal injury—exhibits microscopic capillary hemorrhage.[28] These findings provide some indirect evidence that brain microvascular pericytes provide protection against capillary hemorrhage in the brain.

Functional Characteristics

Vascular endothelium is characterized by constitutive expression of a variety of antithrombotic factors, which are expressed in a tissue-specific manner. In brain microvasculature, the unique cellular configuration impacts endothelial expression of these molecules. Moreover, brain microvascular cells in addition to endothelium, specifically astrocytes and pericytes, seem capable of providing unique contribution to thrombosis and hemostasis.

Functional components of the thrombosis and hemostasis regulatory system involve three principal antithrombotic pathways, as previously described: thrombomodulin, HSPG, and TFPI pathways. Moreover, the endogenous fibrinolytic pathway is tPA-dependent, and the coagulation cascade itself is generated by tissue factor. As will be evident, all these components have expression regulated at the level of the brain microvasculature. Tissue factor and tPA in brain microvasculature are further regulated by specific cellular expression of astrocytes and pericytes.

Thrombomodulin

Thrombomodulin, the endothelial integral membrane protein cofactor for activation of protein C, first attracted neurological attention with the report that it was absent in human brain.[29] This initial observation was followed by a study that demonstrated presence of thrombomodulin in brain capillaries, with particularly low expression in brain regions where small, deep infarctions (lacunes) are most prominent.[30]

These pathological investigations were followed by a series of in vitro studies examining regulation of brain microvascular endothelial thrombomodulin expression. These studies demonstrated transcriptional regulation of endothelial thrombomodulin expression by astrocytes, with ≈20-fold downregulation of thrombomodulin expression when elements of the BBB became manifest.[31] Later work showed that this downregulation was mediated in vitro by transforming growth factor-β.[32] This work was one of the first descriptions of organ-specific regulation of thrombosis and hemostasis.

Recent additional studies of thrombomodulin have shown enhanced expression of thrombomodulin in small arteries in the presence of small vessel disease,[33] raising intriguing possibilities relating thrombomodulin and pathogenesis of small vessel stroke. Whether this enhanced thrombomodulin expression in small vessel disease is specific for brain arteries remains to be determined. The EPCR, located adjacent to thrombomodulin and acting to enhance protein C activation ≈10-fold,[34] has expression preferentially located to endothelium of arteries and veins, with low or absent expression in capillaries of brain and other organs.[35]

Fibrinolytic Pathway: tPA and Plasminogen Activator Inhibitor-1

tPA is the critical endothelial-dependent serine protease, binding to fibrin and activating the fibrinolytic pathway.[4] Capillary tPA expression is largely absent in primate brain, with >95% showing no immunoreactivity.[36] Systemic, but not brain, endothelial cells release tPA in response to α-thrombin in vitro.[37,38] Multiple BBB models have demonstrated restricted expression of brain microvascular endothelial tPA in presence of BBB properties.[39–41]

Plasminogen activator inhibitor-1 (PAI-1) is the principal fibrinolysis inhibitor, and its expression has been studied in vitro and in vivo. Brain expression of PAI-1 shows no overall increased inducible expression in vivo.[42] However, examination of PAI-1 expression in BBB models shows enhanced expression of PAI-1 by brain microvascular endothelium.[39,41,43]

Taken together, these studies of fibrinolysis by brain microvascular endothelium indicate restricted expression

of microvascular tPA and increased expression of PAI-1 by microvascular endothelium. The net effect of these changes is expected to be antifibrinolytic. The specific regulation of brain microvascular fibrinolysis seems to be a function of the BBB.

Tissue Factor

Tissue factor is the principal generator of the coagulation cascade and is known to have a distribution suggesting its function as a hemostatic envelope surrounding blood vessels and encasing organs.[44] The brain, of all body organs, is among the most robust sources of tissue factor.[44,45] Immunohistochemical studies have demonstrated that astrocytes, including BBB astrocytes, are the principal source of tissue factor in the central nervous system.[46] Astrocyte expression of tissue factor at the BBB is entirely consistent with the hemostatic envelope concept, providing protection against hemorrhage particularly at the microvascular level. Tissue factor has also been localized to surface of brain pericytes.[47]

Tissue Factor Pathway Inhibitor

TFPI, representing 1 of 3 critical anticoagulant pathways, is a protease inhibitor synthesized by endothelial cells and acting, via factor Xa, on the tissue factor–VIIa complex.[4] Organ expression study of TFPI demonstrated that only brain demonstrated absence of tissue factor pathway mRNA by Northern blot.[48] Using polymerase chain reaction, TFPI message was detectable in brain and estimated to be at a level ≈1/12 that of lung.[48] Immunohistochemistry demonstrated TFPI protein in brain endothelium; in addition, some staining for TFPI was observed by astrocytes and oligodendrocytes.[48] These overall findings suggest relatively low expression of TFPI by brain.

Antithrombin III–HSPG

Antithrombin III–HSPG represents the third endogenous anticoagulant pathway, in addition to thrombomodulin and TFPI. Antithrombin III is another protease inhibitor, synthesized by liver and forming covalent complexes with coagulation factors, with actions amplified by several orders of magnitude when bound to HSPG in cell membrane or extracellular matrix basement membrane.[49] HSPG is synthesized by endothelial cells, and tissue distribution of HSPG was studied in rat by immunohistochemistry of antithrombin III. Anticoagulantly active HSPG was demonstrable in most organs, but absent in brain capillaries by both light and electron microscopy.[49]

Protease Nexin-1

Protease nexin-1 is a serine protease inhibitor synthesized and secreted by a variety of cell types, including smooth muscle cells and platelets, and capable of inhibiting both thrombin and plasminogen activation.[50] Brain expression of protease nexin-1 has been localized to pericytes in vitro[51] and astrocytes in tissue sections.[52] Given its inhibitory effects on both thrombin and fibrinolysis, the net impact of protease nexin-1 expression (ie, pro- or anticoagulant) in brain is uncertain.

Prostacylin and Endothelial Nitric Oxide

Two critical endogenous regulators of platelet aggregation are prostacyclin and nitric oxide.[53] Prostacyclin is derived from precursor prostaglandin H2 by prostaclyin synthase.[54] Prostacyclin is thought to be largely endothelial-dependent, although neuronal and glial sources, in addition to vascular origin, of prostacyclin synthase have been described.[54] Organ distribution of prostacyclin synthase mRNA has been studied in rat, with relatively low expression in brain compared with most other organs.[55]

Endothelial nitric oxide synthase (eNOS) is the primary nitric oxide synthase expressed by endothelium, with endothelial-derived NO an important regulator of platelet function; other sources of NO (neuronal NOS and inducible NOS) seem to have negligible effects on platelet function.[56] Cell culture studies indicate low expression of eNOS by bovine brain microvascular endothelial cells.[57] However, investigation of eNOS expression in transgenic mice suggested similar expression levels of eNOS compared with other organs studied.[58]

Summary

The brain displays a remarkably consistent pattern of hemostasis regulation, providing a unique system integrating both structural and functional aspects (Table). The presence of tight interendothelial cell junctions, combined with pericyte localization opposite these junctions, is then supplemented with a pattern of underexpression of most anticoagulant factors by endothelial cells, which are then further surrounded by tissue factor–expressing astrocytes. Organ-specific hemostatic regulation, to prevent local hemorrhage, seems to be of exceptional importance for the brain.

Cerebral Microbleeds and Brain-Specific Hemostasis

There has been considerable attention to the phenomenon of cerebral microbleeds during the past decade. These focal areas of hemosiderin iron were initially studied by MRI using gradient echo sequence, and later investigations showed that susceptibility-weighted imaging was even more effective in demonstrating microbleeds.[59] The consensus view is that cerebral microbleeds represent small foci of hemorrhage that are largely age-dependent.[60] In addition to age, principal risk factors for microbleeds are cerebral amyloid angiopathy

Table. Elements of Brain-Specific Regulation of Thrombosis and Hemostasis

	Expression Level/Consequence
Structural components	
Tight junctions	Added barrier to RBC vascular escape
Pericytes	Enhance BBB and provide additional structural barrier to RBC escape
Functional components	
Thrombomodulin	Restricted expression at BBB
Tissue plasminogen activator	Restricted expression at BBB
Plasminogen activator inhibitor-1	Enhanced expression at BBB
Tissue factor	Enhanced expression (by astrocytes) at BBB
Tissue factor pathway inhibitor	Restricted expression at BBB
Heparan sulfate proteoglycans	Restricted expression at BBB

BBB represents blood–brain barrier.

(for cortical microbleeds) and hypertensive vasculopathy (for subcortical lesions).[60,61] Microbleeds limited to lobar location are more prevalent in ApoE €4 carriers and with the ApoE €2/€2 genotype.[60] There is a strong correlation between cerebral microbleeds and cerebral white matter disease of aging (leukoaraiosis),[62–68] and microbleeds are also associated with ischemic and hemorrhagic stroke, as well as Alzheimer disease.[69–71]

Most studies of cerebral microbleeds have focused on their imaging and their correlates with aging and a variety of disease entities as noted above. Increasingly, attention has been directed to what the presence of microbleeds tells us about the brain itself. The latter is an issue that involves neurological consequences of microbleeds, the impact of pharmacotherapies on microbleeds, and perhaps most importantly the underlying pathophysiological mechanisms for cerebral microbleeds.

Clinical and radiological correlates of cerebral microbleeds provide clues to their underlying pathophysiology. (1) Cerebral microbleeds show strong age-dependence. In a population-based cohort study, microbleeds were demonstrable in only 6.5% of subjects aged 45 to 50 years, but increasing to 35.7% in individuals aged ≥80 years.[60] (2) Cerebral amyloid angiopathy is a well-defined risk factor for cerebral microbleeds, particularly cortical or peripheral microbleeds.[60,61] (3) Hypertension is another well-defined risk factor for microbleeds, particularly subcortical or deep microbleeds.[60,61] (4) A strong and consistent association has been observed between cerebral microbleeds and white matter disease of aging.[62–68]

What do these correlates tell us about the process(es) that produces cerebral microbleeds? The current consensus view is that cerebral microbleeds are produced by focal tears in small arteries or arterioles leading to local bleeding,[72,73] and this seems highly likely at least for some cases given the strong association between cerebral microbleeds and clinical intracerebral hemorrhage[74]; indeed, there is evidence suggesting heterogeneity among cerebral microbleeds.[75] Nevertheless, the radiographic appearance of cerebral microbleeds can sometimes suggest a different etiology. Figure 4 shows examples of cerebral microbleeds from 2 different patients in which a diffuse, disseminated process, rather than a focal or multifocal process, would seem to be more likely. These cases, although perhaps clinically extreme, are shown to emphasize a point: a disseminated process seems capable of producing the MRI appearance of cerebral microbleeds.

The likely source of such a diffuse process underlying cerebral microbleeds would involve the microvasculature. There are multiple lines of evidence supporting this contention: (1) White matter disease of aging, strongly correlated with cerebral microbleeds, seems to have a microvascular origin, probably involving the BBB,[76] and is likely to involve inflammatory and oxidative injury.[77] (2) The BBB is well known to exhibit age-dependent changes, with increased permeability demonstrable with aging.[78,79] (3) Both hypertension and cerebral amyloid angiopathy have been shown to contribute independently to BBB dysfunction by oxidative injury, inflammation, and tight junction alterations.[80,81] (4) MRI of high-altitude cerebral edema patients, a clinical syndrome known to be microvascular in origin and almost certainly involving BBB disruption, demonstrates cerebral microbleeds.[82]

Given these elements of circumstantial evidence relating cerebral microbleeds to microvascular dysfunction, it is noteworthy that careful neuropathological evidence has demonstrated age-dependent capillary hemorrhage in human brain.[27,83–86] This was first described as high prevalence of cortical microscopic hemorrhage in aging brain,[83] and high prevalence of capillary-derived cerebral microscopic hemorrhage has subsequently been confirmed by multiple studies.[27,84–86] Indeed, presence of microscopic hemorrhage in putamen is almost invariably present in human brain from subjects aged >70 years and existing independent of either hypertension or local deposition of amyloid.[27]

The fact that there is age-dependent accumulation of small foci of hemorrhage within the brain is striking. As discussed above, the brain is characterized by a unique system of brain-specific hemostasis regulation, focused on the microvasculature, largely encompassed by the BBB, and, by all appearances, designed to protect the brain against occurrences such as microbleeds. It is logical to consider the likelihood that development of cerebral microbleeds represents progressive failure of this system of brain-specific hemostasis, a scenario that is outlined in Figure 5. In this scenario, age-dependent changes of brain-specific hemostasis are amplified by effects of hypertension and amyloid angiopathy, with superimposed transient injury contributing to formation of microhemorrhages/microbleeds. The transient injury may be inflammatory, known to produce enhanced transcellular permeability,[87] and amplification of cerebral microhemorrhages has been demonstrated in this setting.[88]

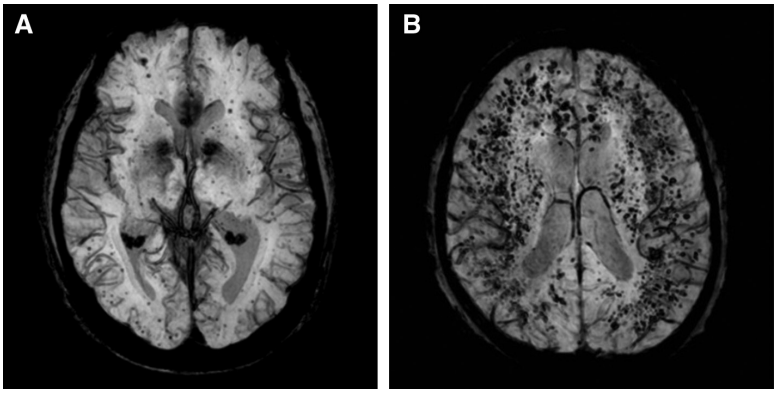

Figure 4. Examples of severe cerebral microbleeds, imaged using 3-Tesla MRI and susceptibility-weighted imaging sequences. **A**, A 67-year-old man with hypertension; (**B**) A 51-year-old man with multiple medical problems, including hypertension, diabetes, end-stage renal disease, sepsis, and thrombocytopenia; a right frontal intracerebral hemorrhage was also present (not shown).

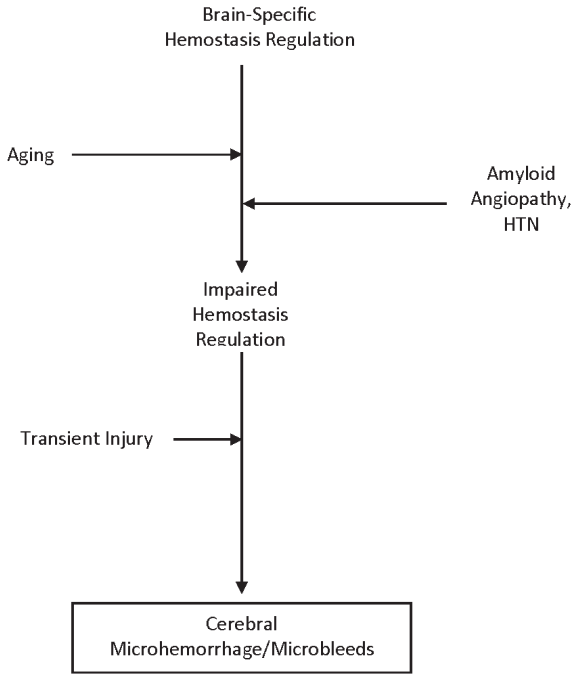

Figure 5. A model relating brain-specific hemostasis regulation to development of cerebral microscopic hemorrhage and microbleeds. Transient injury may be inflammatory. HTN indicates hypertension.

Therapeutic Challenges: Mixed Cerebrovascular Disease

Stroke prevention efforts typically are either–or affairs, focusing on either ischemic stroke or hemorrhagic stroke. It has become increasingly clear that that approach is overly simplistic, due to frequent coexistence of ischemic and hemorrhagic cerebrovascular disease. It thus becomes critical to clarify: For what kind of cerebrovascular disease is preventative treatment being offered?

This difficult clinical context has led to the development of a new diagnostic formulation for stroke: mixed cerebrovascular disease.[89,90] This diagnostic categorization incorporates both ischemic and hemorrhagic stroke, clinical and subclinical. Ischemic syndromes include ischemic stroke (clinical) and silent infarction (subclinical), the latter occurring as much as 4 times more frequently than clinical infarction.[91] White matter disease of aging is included on the ischemic side, with the acknowledged difficulties that it may be sometimes difficult to distinguish normal white matter changes of aging (present in >95% of the population aged >65 years) and pathological white matter disease (leukoariaosis).[92] Hemorrhagic stroke syndromes include intracerebral hemorrhage (clinical) and cerebral microbleeds (subclinical).

The advantages of a diagnostic categorization of mixed cerebrovascular disease are its emphasis on coexistence of ischemic and hemorrhagic processes and the resultant implication that a more specific prevention strategy is indicated. The necessity for this specific prevention approach is based on the substantial body of data indicating that risk of hemorrhagic stroke is predicted by presence of cerebral microbleeds.[74,75,93] Adding to the complexity of this situation are the observations suggesting that microbleeds themselves may contribute to neurological dysfunction (see below), thereby emphasizing the importance of treatment strategies that limit progression of cerebral microbleeds.

Platelet medications used for stroke prevention are known to increase risk for intracerebral hemorrhage, with aspirin increasing risk for hemorrhagic stroke by 84%[94] and combined treatment with aspirin–clopidogrel increasing hemorrhagic stroke risk beyond what is encountered with clopidogrel alone.[95] Multiple reports indicate an important linkage between cerebral microbleeds and risk for intracerebral hemorrhage, for patients with both hypertension and cerebral amyloid angiopathy.[74,75] Cerebral microbleeds, therefore, represent a plausible mechanistic link between use of platelet medications and risk of intracerebral hemorrhage; this has in fact been well demonstrated, when Wong et al[96] and others reported that presence of cerebral microbleeds increased risk for intracerebral hemorrhage in patients using aspirin.[93]

Intracerebral hemorrhage is the most feared complication of anticoagulant therapy, with warfarin therapy carrying a risk of intracerebral hemorrhage generally ranging from 0.3% to 1% annually.[97] This risk is substantially lessened, by as much as 33% to 60%, with the use of the new-generation anticoagulants rivaroxaban and dabigatran.[98,99] Cerebral microbleeds once again represent a plausible mechanistic link, and substantial increased risk of intracerebral hemorrhage (as high as >80-fold increase) has been reported in patients with cerebral microbleeds receiving anticoagulant therapy.[100–102] Moreover, cerebral white matter disease of aging is linked to increased risk (≈13-fold increase) of intracerebral hemorrhage in patients using warfarin,[103] further emphasizing the likely common pathophysiological origin for both white matter disease of aging and cerebral microbleeds.

Although cerebral microbleeds represent an apparent mechanistic link between antithrombotic therapy and risk of intracerebral hemorrhage, the microbleeds themselves are increasingly associated with neurological dysfunction independent of hemorrhage. For example, Qiu et al[104] reported that presence of multiple cerebral microbleeds more than doubled the risk for vascular cognitive impairment. These findings, relating cerebral microbleeds and cognitive impairment, were largely confirmed by later work.[105–107] A relationship between microbleeds and neurological dysfunction is not surprising, given the demonstrable effects of heme on microglial activation via toll-like receptor-4, with resultant enhanced cytokine expression producing inflammatory injury.[108]

Aspirin remains one of the most commonly used platelet medications for stroke prevention, but its use has been implicated in development of cerebral microbleeds. Vernooij et al[109] reported ≈70% increased risk of cerebral microbleeds with use of platelet medications, and chronic use of aspirin (>5 years) was found to be associated with >5-fold increased risk of cerebral microbleeds in a Chinese population treated for cerebrovascular disease.[110] The apparent aspirin–cerebral microbleed linkage, independent of the risk of intracerebral hemorrhage, emphasizes the importance of developing a more refined stroke prevention strategy that is less likely to contribute to development of cerebral microbleeds.

For the patient with mixed cerebrovascular disease, with coexistent ischemic and hemorrhagic processes, what will be

the optimal approach for preventative treatment? Given the observed associations linking platelet medications and anticoagulants with microbleeds and intracerebral hemorrhage, it is apparent that the standard one-size-fits-all approach for stroke prevention is insufficient. For the present, perhaps the most attractive strategy involves combining platelet effects with vascular wall protection.

A simple approach to achieve platelet inhibition and vessel wall protection is via modulation of intracellular cyclic nucleotides, for both platelets and vascular endothelial cells. This is achievable via modification of signal transduction pathways using phosphodiesterase (PDE) inhibitors to regulate intracellular levels of cAMP and cGMP. PDE inhibitors, of which there are 11 families with >60 isoforms, modulate hydrolysis of these cyclic nucleotides.[111] Elevation of platelet cAMP and cGMP interferes with all known platelet activation pathways,[111] whereas cAMP pathways have a critical role in development of the BBB.[112] Both dipyridamole and cilostazol are PDE inhibitors that have already been studied in stroke prevention trials and shown to be beneficial.[113–116]

Dipyridamole, with stroke prevention effects comparable to aspirin,[113] acts via relatively nonspecific PDE inhibition impacting both PDE3 and PDE5.[111] Dipyridamole produces platelet effects via elevation of plasma adenosine (by reduced red cell uptake) and increasing effects of prostacyclin and nitric oxide, whereas vessel wall protection is achieved by antioxidative effects and reduction of interactions between platelets and monocytes.[117] Reduction of infarct size in experimental stroke has been demonstrated with dipyridamole.[118] Moreover, in a mouse model of cerebral microbleeds and at clinically relevant plasma levels, dipyridamole did not worsen cerebral microscopic hemorrhage in aged transgenic animals subjected to immunotherapy-induced hemorrhagic worsening.[88]

Cilostazol is a specific PDE3 inhibitor, resulting in relatively selective inhibition of cAMP hydrolysis.[111] Cilostazol inhibits multiple pathways of platelet activation and aggregation, whereas vessel wall protection has been demonstrated in vitro by enhancement of endothelial cell barrier properties and reduction of histamine-induced transient barrier disruption.[111,119] Cilostazol has also been shown to reduce hemorrhagic conversion in several murine models of experimental stroke.[120,121] In clinical stroke prevention trials, cilostazol has been demonstrated as effective compared with both placebo and aspirin. Hemorrhagic events, including intracerebral hemorrhage, were reduced by more than one half for cilostazol, compared with aspirin treatment.[116]

The patient with mixed cerebrovascular disease presents a unique therapeutic challenge, in which both ischemic risk and hemorrhagic tendencies must be addressed simultaneously. Therapy concurrently directed to both platelets and the vessel wall seems to be an attractive way to address this dilemma. This therapeutic challenge thus appears to represent a consequence of changes in the specific system of hemostasis regulation that resides in the brain.

Conclusions

The unique hemostasis regulatory system present in the brain resides in the microvasculature and seems primed to protect the brain against hemorrhagic injury. This regulatory system has important structural and functional components and appears largely to be a component of the BBB or neurovascular unit. It is proposed that changes in brain-specific hemostasis regulation are a critical underlying factor for age-dependent hemorrhagic changes of the brain, which manifest pathologically as microscopic hemorrhage and radiographically as cerebral microbleeds.

Much work needs to be done to fully flesh out this conceptual framework. Development of new animal models of cerebral microbleeds will be an important step,[122] as well as definition of molecular elements of age-dependent changes of the BBB. Exquisitely careful correlations will be necessary between MRI cerebral microbleeds and neuropathologically demonstrable cerebral microscopic hemorrhage. The relationship between cerebral microinfarcts[123] and cerebral microscopic hemorrhage[86] represents an important area for investigation, particularly with potential contribution of hemorrhagic microinfarction to development of cerebral microbleeds.[86]

The future evolution of stroke prevention efforts will need to more carefully address the underlying pathophysiology of the ongoing cerebrovascular processes of concern. Just as with the revision of our concepts of transient ischemic attacks,[124] newer definitions such as mixed cerebrovascular disease may lead to more effective efforts to reduce the prevalence of stroke. This will only come about with optimal efforts addressing stroke prevention.

Acknowledgments

The author thanks Cheryl Cotman for assistance.

Sources of Funding

This study was supported by National Institutes of Health RO1 NS20989.

Disclosures

Dr Fisher has received research support from Boehringer-Ingelheim and Otsuka Pharmaceutical Company.

References

1. Aird WC. Coagulation. *Crit Care Med*. 2005;33(12 suppl):S485–S487.
2. del Zoppo GJ. Virchow's triad: the vascular basis of cerebral injury. *Rev Neurol Dis*. 2008;5(suppl 1):S12–S21.
3. Wolberg AS, Aleman MM, Leiderman K, Machlus KR. Procoagulant activity in hemostasis and thrombosis: Virchow's triad revisited. *Anesth Analg*. 2012;114:275–285.
4. Mackman N. Tissue-specific hemostasis in mice. *Arterioscler Thromb Vasc Biol*. 2005;25:2273–2281.
5. Rosenberg RD, Aird WC. Vascular-bed–specific hemostasis and hypercoagulable states. *N Engl J Med*. 1999;340:1555–1564.
6. Aird WC. Vascular bed-specific thrombosis. *J Thromb Haemost*. 2007;5(suppl 1):283–291.
7. Osterud B, Bajaj MS, Bajaj SP. Sites of tissue factor pathway inhibitor (TFPI) and tissue factor expression under physiologic and pathologic conditions. On behalf of the Subcommittee on Tissue factor Pathway Inhibitor (TFPI) of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost*. 1995;73:873–875.
8. Yamamoto K, de Waard V, Fearns C, Loskutoff DJ. Tissue distribution and regulation of murine von Willebrand factor gene expression in vivo. *Blood*. 1998;92:2791–2801.
9. Lurie F, Kistner RL, Eklof B, Kessler D. Mechanism of venous valve closure and role of the valve in circulation: a new concept. *J Vasc Surg*. 2003;38:955–961.

10. Primignani M, Martinelli I, Bucciarelli P, Battaglioli T, Reati R, Fabris F, et al. Risk factors for thrombophilia in extrahepatic portal vein obstruction. *Hepatology*. 2005;41:603–608.
11. Michiels JJ, Berneman ZN, Schroyens W, Van Vliet HH. Pathophysiology and treatment of platelet-mediated disturbances, major thrombosis and bleeding complications in essential thrombocythaemia and poycythaemia vera. *Platelets*. 2004;15:67–84.
12. Comp PC, Elrod JP, Karzenski S. Warfarin-induced skin necrosis. *Semin Thromb Hemost*. 1990;16:293–298.
13. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degen J, Bronson R, et al. Physiological consequences of loss of plasminogen activator gene function in mice. *Nature*. 1994;368:419–424.
14. Weiler-Guettler H, Christie PD, Beeler DL, Healy AM, Hancock WW, Rayburn H, et al. A targeted point mutation in thrombomodulin generates viable mice with a prethrombotic state. *J Clin Invest*. 1998;101:1983–1991.
15. Healy AM, Hancock WW, Christie PD, Rayburn HB, Rosenberg RD. Intravascular coagulation activation in a murine model of thrombomodulin deficiency: effects of lesion size, age, and hypoxia on fibrin deposition. *Blood*. 1998;92:4188–4197.
16. Weiler H, Lindner V, Kerlin B, Isermann BH, Hendrickson SB, Cooley BC, et al. Characterization of a mouse model for thrombomodulin deficiency. *Arterioscler Thromb Vasc Biol*. 2001;21:1531–1537.
17. Christie PD, Edelberg JM, Picard MH, Foulkes AS, Mamuya W, Weiler-Guettler H, et al. A murine model of myocardial microvascular thrombosis. *J Clin Invest*. 1999;104:533–539.
18. Maroney SA, Cooley BC, Sood R, Weiler H, Mast AE. Combined tissue factor pathway inhibitor and thrombomodulin deficiency produces an augmented hypercoagulable state with tissue-specific fibrin deposition. *J Thromb Haemost*. 2008;6:111–117.
19. Kim JH, Kim JH, Park JA, Lee SW, Kim WJ, Yu YS, et al. Blood-neural barrier: intercellular communication at glio-vascular interface. *J Biochem Mol Biol*. 2006;39:339–345.
20. Nachman RL, Rafii S. Platelets, petechiae, and preservation of the vascular wall. *N Engl J Med*. 2008;359:1261–1270.
21. Butros LJ, Bussel JB. Intracranial hemorrhage in immune thrombocytopenic purpura: a retrospective analysis. *J Pediatr Hematol Oncol*. 2003;25:660–664.
22. Liu S, Agalliu D, Yu C, Fisher M. The role of pericytes in blood-brain barrier function and stroke. *Curr Pharm Des*. 2012;18:3653–3662.
23. Maxwell K, Berliner JA, Cancilla PA. Induction of gamma-glutamyl transpeptidase in cultured cerebral endothelial cells by a product released by astrocytes. *Brain Res*. 1987;410:309–314.
24. Al Ahmad A, Taboada CB, Gassmann M, Ogunshola OO. Astrocytes and pericytes differentially modulate blood-brain barrier characteristics during development and hypoxic insult. *J Cereb Blood Flow Metab*. 2011;31:693–705.
25. Bonerandi JJ, Bourdet D, Privat Y, Temime P. Kaposi's disease: ultrastructural study of 4 cases. *Ann Dermatol Syphilligr (Paris)*. 1975;102:499–506.
26. Schenk P. Erythrophagocytosis in Kaposi's sarcoma in acquired immune deficiency syndrome. *ORL*. 1986;48:167–173.
27. Fisher M, French S, Ji P, Kim RC. Cerebral microbleeds in the elderly: a pathological analysis. *Stroke*. 2010;41:2782–2785.
28. Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, et al. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron*. 2010;68:409–427.
29. Ishii H, Salem HH, Bell CE, Laposata EA, Majerus PW. Thrombomodulin, an endothelial anticoagulant protein, is absent from the human brain. *Blood*. 1986;67:362–365.
30. Wong VL, Hofman FM, Ishii H, Fisher M. Regional distribution of thrombomodulin in human brain. *Brain Res*. 1991;556:1–5.
31. Tran N, Wong VLY, Schreiber SS, Bready J, Fisher M. Regulation of brain capillary endothelial thrombomodulin mRNA expression. *Stroke*. 1996;27:2304–2311.
32. Tran ND, Correale J, Schreiber SS, Fisher M. Transforming growth factor-beta mediates astrocyte-specific regulation of brain endothelial anticoagulant factors. *Stroke*. 1999;30:1671–1678.
33. Giwa MO, Williams J, Elderfield K, Jiwa NS, Bridges LR, Kalaria RN, et al. Neuropathologic evidence of endothelial changes in cerebral small vessel disease. *Neurology*. 2012;78:167–174.
34. Montes R, Puy C, Molina E, Hermida J. Is EPCR a multi-ligand receptor? Pros and cons. *Thromb Haemost*. 2012;107:815–826.
35. Laszik Z, Mitro A, Taylor FB Jr, Ferrell G, Esmon CT. Human protein C receptor is present primarily on endothelium of large blood vessels: implications for the control of the protein C pathway. *Circulation*. 1997;96:3633–3640.
36. Levin EG, del Zoppo GJ. Localization of tissue plasminogen activator in the endothelium of a limited number of vessels. *Am J Pathol*. 1994;144:855–861.
37. Shatos MA, Orfeo T, Doherty JM, Penar PL, Collen D, Mann KG. Alpha-thrombin stimulates urokinase production and DNA synthesis in cultured human cerebral microvascular endothelial cells. *Arterioscler Thromb Vasc Biol*. 1995;15:903–911.
38. Grau GE, de Moerloose P, Bulla O, Lou J, Lei Z, Reber G, et al. Haemostatic properties of human pulmonary and cerebral microvascular endothelial cells. *Thromb Haemost*. 1997;77:585–590.
39. Tran ND, Schreiber SS, Fisher M. Astrocyte regulation of endothelial tissue plasminogen activator in a blood-brain barrier model. *J Cereb Blood Flow Metab*. 1998;18:1316–1324.
40. Kim JA, Wang SJ, Fisher MJ. Astrocyte regulation of human brain capillary endothelial fibrinolysis. *Thromb Res*. 2003;112:159–165.
41. Yang F, Liu S, Wang SJ, Yu C, Paganini-Hill A, Fisher MJ. Tissue plasminogen activator expression and barrier properties of human brain microvascular endothelial cells. *Cell Physiol Biochem*. 2011;28:631–638.
42. Sawdey MS, Loskutoff DJ. Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. *J Clin Invest*. 1991;88:1346–1353.
43. Hultman K, Björklund U, Hansson E, Jern C. Potentiating effect of endothelial cells on astrocytic plasminogen activator inhibitor type-1 gene expression in an in vitro model of the blood-brain barrier. *Neuroscience*. 2010;166:408–415.
44. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues: Implications for disorders of hemostasis and thrombosis. *Am J Pathol*. 1989;134:1087–1097.
45. Fleck RA, Rao LJM, Rapaport SI, Varki N. Localization of human tissue factor antigen by immunostaining with monspecific, polyclonal anti-human tissue factor antibody. *Thromb Res*. 1990;57:765–782.
46. Eddleston M, de la Torre JC, Oldstone MB, Loskutoff DJ, Edgington TS, Mackman N. Astrocytes are the primary source of tissue factor in the murine central nervous system. A role for astrocytes in cerebral hemostasis. *J Clin Invest*. 1993;92:349–358.
47. Bouchard BA, Shatos MA, Tracy PB. Human brain pericytes differentially regulate expression of procoagulant enzyme complexes comprising the extrinsic pathway of blood coagulation. *Arterioscler Thromb Vasc Biol*. 1997;17:1–9.
48. Bajaj MS, Kuppuswamy MN, Manepalli AN, Bajaj SP. Transcriptional expression of tissue factor pathway inhibitor, thrombomodulin and von Willebrand factor in normal human tissues. *Thromb Haemost*. 1999;82:1047–1052.
49. Xu Y, Slayter HS. Immunocytochemical localization of endogenous antithrombin III in the vasculature of rat tissues reveals locations of anticoagulantly active heparan sulfate proteoglycans. *J Histochem Cytochem*. 1994;42:1365–1376.
50. Richard B, Arocas V, Guillin MC, Michel JB, Jandrot-Perrus M, Bouton MC. Protease nexin-1: a cellular serpin down-regulated by thrombin in rat aortic smooth muscle cells. *J Cell Physiol*. 2004;201:138–145.
51. Kim JA, Tran ND, Li Z, Yang F, Zhou W, Fisher MJ. Brain endothelial hemostasis regulation by pericytes. *J Cereb Blood Flow Metab*. 2006;26:209–217.
52. Choi BH, Suzuki M, Kim T, Wagner SL, Cunningham DD. Protease nexin-1. Localization in the human brain suggests a protective role against extravasated serine proteases. *Am J Pathol*. 1990;137:741–747.
53. Broos K, Feys HB, De Meyer SF, Vanhoorelbeke K, Deckmyn H. Platelets at work in primary hemostasis. *Blood Rev*. 2011;25:155–167.
54. Siegle I, Klein T, Zou MH, Fritz P, Kömhoff M. Distribution and cellular localization of prostacyclin synthase in human brain. *J Histochem Cytochem*. 2000;48:631–641.
55. Tone Y, Inoue H, Hara S, Yokoyama C, Hatae T, Oida H, et al. The regional distribution and cellular localization of mRNA encoding rat prostacyclin synthase. *Eur J Cell Biol*. 1997;72:268–277.
56. Moore C, Sanz-Rosa D, Emerson M. Distinct role and location of the endothelial isoform of nitric oxide synthase in regulating platelet aggregation in males and females in vivo. *Eur J Pharmacol*. 2011;651:152–158.
57. Kimura C, Oike M, Ohnaka K, Nose Y, Ito Y. Constitutive nitric oxide production in bovine aortic and brain microvascular endothelial cells: a comparative study. *J Physiol*. 2004;554(pt 3):721–730.
58. Guillot PV, Guan J, Liu L, Kuivenhoven JA, Rosenberg RD, Sessa WC, et al. A vascular bed-specific pathway regulates cardiac expression of endothelial nitric oxide synthase. *J Clin Invest*. 1999;103:799–805.

59. Nandigam RN, Viswanathan A, Delgado P, Skehan ME, Smith EE, Rosand J, et al. MR imaging detection of cerebral microbleeds: effect of susceptibility-weighted imaging, section thickness, and field strength. *AJNR Am J Neuroradiol*. 2009;30:338–343.
60. Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, et al. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. *Stroke*. 2010;41(10 suppl):S103–S106.
61. Greenberg SM. Cerebral amyloid angiopathy: prospects for clinical diagnosis and treatment. *Neurology*. 1998;51:690–694.
62. Kato H, Izumiyama M, Izumiyama K, Takahashi A, Itoyama Y. Silent cerebral microbleeds on T2*-weighted MRI: correlation with stroke subtype, stroke recurrence, and leukoaraiosis. *Stroke*. 2002;33:1536–1540.
63. Lee SH, Bae HJ, Ko SB, Kim H, Yoon BW, Roh JK. Comparative analysis of the spatial distribution and severity of cerebral microbleeds and old lacunes. *J Neurol Neurosurg Psychiatry*. 2004;75:423–427.
64. Naka H, Nomura E, Wakabayashi S, Kajikawa H, Kohriyama T, Mimori Y, et al. Frequency of asymptomatic microbleeds on T2*-weighted MR images of patients with recurrent stroke: association with combination of stroke subtypes and leukoaraiosis. *AJNR Am J Neuroradiol*. 2004;25:714–719.
65. Maia LF, Vasconcelos C, Seixas S, Magalhães R, Correia M. Lobar brain hemorrhages and white matter changes: clinical, radiological and laboratorial profiles. *Cerebrovasc Dis*. 2006;22:155–161.
66. Görner A, Lemmens R, Schrooten M, Thijs V. Is leukoaraiosis on CT an accurate surrogate marker for the presence of microbleeds in acute stroke patients? *J Neurol*. 2007;254:284–289.
67. Gao T, Wang Y, Zhang Z. Silent cerebral microbleeds on susceptibility-weighted imaging of patients with ischemic stroke and leukoaraiosis. *Neurol Res*. 2008;30:272–276.
68. Yamada S, Saiki M, Satow T, Fukuda A, Ito M, Minami S, et al. Periventricular and deep white matter leukoaraiosis have a closer association with cerebral microbleeds than age. *Eur J Neurol*. 2012;19:98–104.
69. Lee SH, Heo JH, Yoon BW. Effects of microbleeds on hemorrhage development in leukoaraiosis patients. *Hypertens Res*. 2005;28:895–899.
70. Pettersen JA, Sathiyamoorthy G, Gao FQ, Szilagyi G, Nadkarni NK, St George-Hyslop P, et al. Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. *Arch Neurol*. 2008;65:790–795.
71. Jeon SB, Kwon SU, Cho AH, Yun SC, Kim JS, Kang DW. Rapid appearance of new cerebral microbleeds after acute ischemic stroke. *Neurology*. 2009;73:1638–1644.
72. Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, et al. Histopathologic analysis of foci of signal loss on gradient-echo T2*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. *AJNR Am J Neuroradiol*. 1999;20:637–642.
73. Schrag M, McAuley G, Pomakian J, Jiffry A, Tung S, Mueller C, et al. Correlation of hypointensities in susceptibility-weighted images to tissue histology in dementia patients with cerebral amyloid angiopathy: a postmortem MRI study. *Acta Neuropathol*. 2010;119:291–302.
74. Fan YH, Zhang L, Lam WW, Mok VC, Wong KS. Cerebral microbleeds as a risk factor for subsequent intracerebral hemorrhages among patients with acute ischemic stroke. *Stroke*. 2003;34:2459–2462.
75. Greenberg SM, Nandigam RN, Delgado P, Betensky RA, Rosand J, Viswanathan A, et al. Microbleeds versus macrobleeds: evidence for distinct entities. *Stroke*. 2009;40:2382–2386.
76. Young VG, Halliday GM, Kril JJ. Neuropathologic correlates of white matter hyperintensities. *Neurology*. 2008;71:804–811.
77. Back SA, Kroenke CD, Sherman LS, Lawrence G, Gong X, Taber EN, et al. White matter lesions defined by diffusion tensor imaging in older adults. *Ann Neurol*. 2011;70:465–476.
78. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease–systematic review and meta-analysis. *Neurobiol Aging*. 2009;30:337–352.
79. Popescu BO, Toescu EC, Popescu LM, Bajenaru O, Muresanu DF, Schultzberg M, et al. Blood-brain barrier alterations in ageing and dementia. *J Neurol Sci*. 2009;283:99–106.
80. Pires PW, Dams Ramos CM, Matin N, Dorrance AM. The effects of hypertension on the cerebral circulation. *Am J Physiol Heart Circ Physiol*. 2013;304:H1598–H1614.
81. Carrano A, Hoozemans JJ, van der Vies SM, van Horssen J, de Vries HE, Rozemuller AJ. Neuroinflammation and blood-brain barrier changes in capillary amyloid angiopathy. *Neurodegener Dis*. 2012;10:329–331.
82. Kallenberg K, Dehnert C, Dörfler A, Schellinger PD, Bailey DM, Knauth M, et al. Microhemorrhages in nonfatal high-altitude cerebral edema. *J Cereb Blood Flow Metab*. 2008;28:1635–1642.
83. Cullen KM, Kócsi Z, Stone J. Pericapillary haem-rich deposits: evidence for microhaemorrhages in aging human cerebral cortex. *J Cereb Blood Flow Metab*. 2005;25:1656–1667.
84. Cullen KM, Kócsi Z, Stone J. Microvascular pathology in the aging human brain: evidence that senile plaques are sites of microhaemorrhages. *Neurobiol Aging*. 2006;27:1786–1796.
85. De Reuck J, Deramecourt V, Cordonnier C, Leys D, Pasquier F, Maurage CA. Prevalence of small cerebral bleeds in patients with a neurodegenerative dementia: a neuropathological study. *J Neurol Sci*. 2011;300:63–66.
86. Tanskanen M, Mäkelä M, Myllykangas L, Rastas S, Sulkava R, Paetau A. Intracerebral hemorrhage in the oldest old: a population-based study (vantaa 85+). *Front Neurol*. 2012;3:103.
87. Kumar P, Shen Q, Pivetti CD, Lee ES, Wu MH, Yuan SY. Molecular mechanisms of endothelial hyperpermeability: implications in inflammation. *Expert Rev Mol Med*. 2009;11:e19.
88. Fisher M, Vasilevko V, Passos GF, Ventura C, Quiring D, Cribbs DH. Therapeutic modulation of cerebral microhemorrhage in a mouse model of cerebral amyloid angiopathy. *Stroke*. 2011;42:3300–3303.
89. Fisher M. The challenge of mixed cerebrovascular disease. *Ann N Y Acad Sci*. 2010;1207:18–22.
90. Fisher M, Vasilevko V, Cribbs DH. Mixed cerebrovascular disease and the future of stroke prevention. Transl. *Stroke Res*. 2012;3 (suppl 1):S39–S51.
91. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. *Stroke*. 2002;33:21–25.
92. Longstreth WT Jr, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, et al. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. *Stroke*. 1996;27:1274–1282.
93. Biffi A, Halpin A, Towfighi A, Gilson A, Busl K, Rost N, et al. Aspirin and recurrent intracerebral hemorrhage in cerebral amyloid angiopathy. *Neurology*. 2010;75:693–698.
94. He J, Whelton PK, Vu B, Klag MJ. Aspirin and risk of hemorrhagic stroke: a meta-analysis of randomized controlled trials. *JAMA*. 1998;280:1930–1935.
95. Diener HC, Bogousslavsky J, Brass LM, Cimminiello C, Csiba L, Kaste M, et al; MATCH Investigators. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. *Lancet*. 2004;364:331–337.
96. Wong KS, Chan YL, Liu JY, Gao S, Lam WW. Asymptomatic microbleeds as a risk factor for aspirin-associated intracerebral hemorrhages. *Neurology*. 2003;60:511–513.
97. Flaherty ML. Anticoagulant-associated intracerebral hemorrhage. *Semin Neurol*. 2010;30:565–572.
98. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, et al; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139–1151.
99. Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, et al; ROCKET AF Investigators. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med*. 2011;365:883–891.
100. Ueno H, Naka H, Ohshita T, Kondo K, Nomura E, Ohtsuki T, et al. Association between cerebral microbleeds on T2*-weighted MR images and recurrent hemorrhagic stroke in patients treated with warfarin following ischemic stroke. *AJNR Am J Neuroradiol*. 2008;29:1483–1486.
101. Lee SH, Ryu WS, Roh JK. Cerebral microbleeds are a risk factor for warfarin-related intracerebral hemorrhage. *Neurology*. 2009;72:171–176.
102. Lovelock CE, Cordonnier C, Naka H, Al-Shahi Salman R, Sudlow CL, Sorimachi T, et al; Edinburgh Stroke Study Group. Antithrombotic drug use, cerebral microbleeds, and intracerebral hemorrhage: a systematic review of published and unpublished studies. *Stroke*. 2010;41:1222–1228.
103. Smith EE, Rosand J, Knudsen KA, Hylek EM, Greenberg SM. Leukoaraiosis is associated with warfarin-related hemorrhage following ischemic stroke. *Neurology*. 2002;59:193–197.
104. Qiu C, Cotch MF, Sigurdsson S, Jonsson PV, Jonsdottir MK, Sveinbjrnsdottir S, et al. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology*. 2010;75:2221–2228.
105. Takashima Y, Mori T, Hashimoto M, Kinukawa N, Uchino A, Yuzuriha T, et al. Clinical correlating factors and cognitive function in community-dwelling healthy subjects with cerebral microbleeds. *J Stroke Cerebrovasc Dis*. 2011;20:105–110.
106. van Es AC, van der Grond J, de Craen AJ, Westendorp RG, Bollen EL, Blauw GJ, et al; PROSPER Study Group. Cerebral microbleeds and cognitive functioning in the PROSPER study. *Neurology*. 2011;77:1446–1452.

107. Poels MM, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, et al. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology*. 2012;78:326–333.
108. Lin S, Yin Q, Zhong Q, Lv FL, Zhou Y, Li JQ, et al. Heme activates TLR4-mediated inflammatory injury via MyD88/TRIF signaling pathway in intracerebral hemorrhage. *J Neuroinflammation*. 2012;9:46.
109. Vernooij MW, Haag MD, van der Lugt A, Hofman A, Krestin GP, Stricker BH, et al. Use of antithrombotic drugs and the presence of cerebral microbleeds: the Rotterdam Scan Study. *Arch Neurol*. 2009;66: 714–720.
110. Ge L, Niu G, Han X, Gao Y, Wu Q, Wu H, et al. Aspirin treatment increases the risk of cerebral microbleeds. *Can J Neurol Sci*. 2011;38:863–868.
111. Gresele P, Momi S, Falcinelli E. Anti-platelet therapy: phosphodiesterase inhibitors. *Br J Clin Pharmacol*. 2011;72:634–646.
112. Rubin LL, Hall DE, Porter S, Barbu K, Cannon C, Horner HC, et al. A cell culture model of the blood-brain barrier. *J Cell Biol*. 1991;115:1725–1735.
113. Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European Stroke Prevention Study. 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. *J Neurol Sci*. 1996; 143:1–13.
114. Gotoh F, Tohgi H, Hirai S, Terashi A, Fukuuchi Y, Otomo E, et al. Cilostazol stroke prevention study: A placebo controlled double-blind trial for secondary prevention of cerebral infarction. *J Stroke Cerebrovasc Dis*. 2000;9:147–157.
115. Huang Y, Cheng Y, Wu J, Li Y, Xu E, Hong Z, et al; Cilostazol versus Aspirin for Secondary Ischaemic Stroke Prevention Cooperation Investigators. Cilostazol as an alternative to aspirin after ischaemic stroke: a randomised, double-blind, pilot study. *Lancet Neurol*. 2008;7:494–499.
116. Shinohara Y, Katayama Y, Uchiyama S, Yamaguchi T, Handa S, Matsuoka K, et al; CSPS 2 Group. Cilostazol for prevention of secondary stroke (CSPS 2): an aspirin-controlled, double-blind, randomised non-inferiority trial. *Lancet Neurol*. 2010;9:959–968.
117. Kim HH, Liao JK. Translational therapeutics of dipyridamole. *Arterioscler Thromb Vasc Biol*. 2008;28:s39–s42.
118. García-Bonilla L, Sosti V, Campos M, Penalba A, Boada C, Sumalla M, et al. Effects of acute post-treatment with dipyridamole in a rat model of focal cerebral ischemia. *Brain Res*. 2011;1373:211–220.
119. Liu S, Yu C, Yang F, Paganini-Hill A, Fisher MJ. Phosphodiesterase inhibitor modulation of brain microvascular endothelial cell barrier properties. *J Neurol Sci*. 2012;320:45–51.
120. Hase Y, Okamoto Y, Fujita Y, Kitamura A, Nakabayashi H, Ito H, et al. Cilostazol, a phosphodiesterase inhibitor, prevents no-reflow and hemorrhage in mice with focal cerebral ischemia. *Exp Neurol*. 2012;233:523–533.
121. Kasahara Y, Nakagomi T, Matsuyama T, Stern D, Taguchi A. Cilostazol reduces the risk of hemorrhagic infarction after administration of tissue-type plasminogen activator in a murine stroke model. *Stroke*. 2012;43:499–506.
122. Liu S, Vasilevko V, Cribbs DH, Fisher M. A mouse model of cerebral microbleeds. *Stroke*. 2013;44:AWP297.
123. Westover MB, Bianchi MT, Yang C, Schneider JA, Greenberg SM. Estimating cerebral microinfarct burden from autopsy samples. *Neurology*. 2013;80:1365–1369.
124. Easton JD, Saver JL, Albers GW, Alberts MJ, Chaturvedi S, Feldmann E, et al. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/ American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. *Stroke*. 2009;40:2276–2293.

KEY WORDS: blood-brain barrier ■ hemorrhage ■ hemostasis ■ thrombosis