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The Brain Hemostasis: Implications for Stroke Pathology and New Therapeutic Strategies

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Abstract

It is well documented that the hemostatic response has been shown to vary between organs and blood vessels. The brain is the organ that expresses the unique hemostasis regulatory system, which may be crucial for understanding of the stroke pathology and cerebral hemorrhage. Previously, it was perceived that the brain comparing to other organs, has been functioning based less on antithrombotic and fibrinolytic pathways while protection against hemorrhage was a higher priority for this organ. At present, more attention has been focused on the brain prothrombotic potential predisposing to brain infarction. Therefore, the relationship between cerebral microinfarcts and microscopic hemorrhages represents an important area for investigation. The understanding of the brain heterogenecity of hemostatic mechanisms may provide a new approach for specific prediction, prevention and treatment of thrombotic brain damage.

This paper will focus on main regulators of brain hemostasis: endothelial cells with endothelium-derived hemostatic factors and neurovascular unit with pericytes and astrocytes. The paper will address also the role of contact-kinin pathway in the thromo inflammatory pathology of ischemic stroke, as well as the role of amyloid β-protein precursor in the brain hemostasis regulation.

Introduction

During the past decades, many studies were dedicated to the mechanisms underlying ischemic stroke and cerebral reperfusion injury. Brain locally regulated hemostasis may be of the key importance for understanding of this pathology. It has been also generally accepted that both thrombotic and inflammatory pathways are important pathophysiologic contributors to ischemic stroke. At vascular lesions, blood platelets are activated, increasing the risk of secondary thrombotic events. At the same time, cerebral ischemia elicits a strong inflammatory response involving among others upregulation of cell adhesion molecules and cytokines as well as adhesion, activation, and transmigration of leukocytes [1,2]. An important link between these thrombotic and inflammatory pathways in stroke leads to the concept of thromboinflammation in the stroke etiology constituting a big therapeutic challenge [3,4].

Hemostasis – Cells Controlled Coagulation Process

According to the current knowledge, coagulation model can be divided into three separate phases: 1) initiation phase, in which low amounts of active coagulant factors are generated; 2) amplification phase, in which the level of active coagulation factors is boosted and 3) propagation phase, in which coagulation factors bind to highly pro coagulant membranes of activated platelets and fiber in clots are formed. This concept of hemostasis assumed the following scenario. Upon endothelial damage, tissue factor (TF) is exposed to the blood stream and binds factor VII, which is activated to factor VIIa. The TF: VIIa complex enables subsequent activation of factor X and prothrombin, after which small amount of thrombin activates the factor XI-IX feedback loop on the platelet surface. Factor IXa activates additional factor X. Simultaneously, the trace amount of thrombin will then activate factors VIII (cofactor to factor IX) and V (cofactor to factor X), which dramatically enhances catalytic activity of factors IX and X. Finally, thrombin (factor IIa) activation leads to fibr in deposition. In parallel, local polyphosphate (Poly P) release by activated platelets may additionally stimulate activation of factor XII, factor V, and FXI and inhibit clot lysis [5,6].

This so called cell biological model of coagulation is gaining attention though more classical division between intrinsic – factor XII-activated pathway and extrinsic – TF-activated pathway is still in use. The natural inactivation of coagulation process involves circulating protease inhibitors, such

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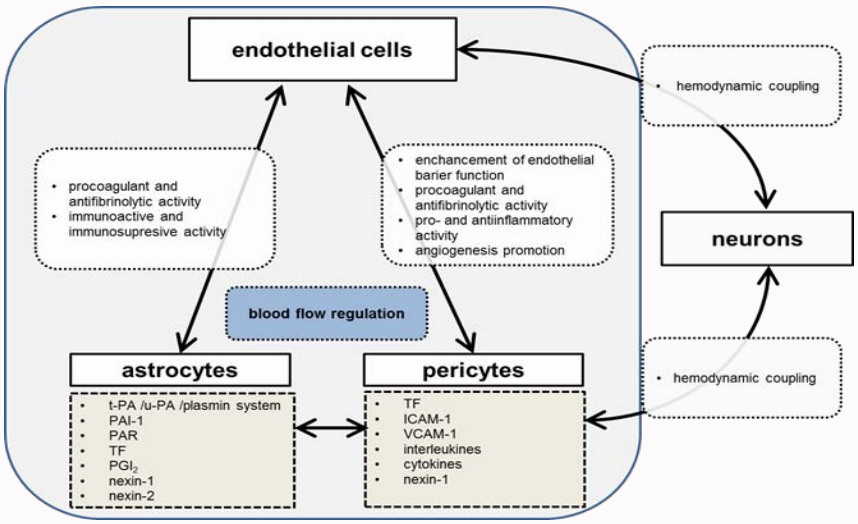

Figure 1: Neurovascular units as a regulator of brain hemostasis.

as antithrombin, heparin cofactor II, tissue factor pathway inhibitor (TFPI), and C1 esterase inhibitor. They eliminate activated factors by attaching their active sites. The second anticoagulant pathway is provided by the enzyme-based protein C/protein S activity. The latter is implicated in endothelial-based pathways of coagulation inactivation [6].

Vascular Bed Specific Hemostasis

The majority of factors in the coagulation as well as fibrinolytic cascade are generated by the liver and secreted into the circulation. Similarly, platelets, erythrocytes and leukocytes circulating in the blood are central to the process of clotting. The function of the local vasculature depends mainly on the endothelial cells (ECs) and endothelium-derived factors. The interaction between the systemic and local pathways provides the necessary action required to regulate vascular bed-specific hemostatic activity.

It is well known that endothelium plays a crucial role in maintaining blood in a fluidic state and promoting limited clot formation in a case of interruption of the vascular wall integrity. ECc express TFPI, heparin, thrombomodulin (TM), endothelial protein C receptor (EPCR), receptors of plasminogen (annexin II) and urokinase, as well as the secretion of tissue-type and urokinase plasminogen activators (t-PA, u-PA), ecto-ADPase, nitric oxide (NO), prostacyclin (PGI2); all relevant for anticoagulant activity. On the procoagulant side, ECs synthesize plasminogen activator inhibitor (PAI-1), von Willebrand factor (v WF), and protease activated receptors [5,6].

Importantly, endothelial-derived anticoagulant and procoagulant molecules are unequally distributed throughout the vascular bed. A growing body of evidence suggests extensive heterogeneity among endothelial cells *in vivo*. The differential distribution of procoagulants and anticoagulants in the endothelium suggests that ECs from different locations of the vascular tree use site-specific "code" of procoagulants and anticoagulants to balance local hemostasis. There is an evidence that congenital or acquired alterations in the dynamic balance can be marked in vascular pathophysiology by bleeding or hypercoagulability. Then, the hypercoagulable state may lead to thrombosis. It was demonstrated that disorders observed in hemostasis may be associated with distinct vascular beds, thus implying that the relative combined contribution of individual regulatory pathways may be specific and/or unique to a particular site in the vasculature. This diversity reflects the interaction of systemic and endothelial components in hemostatic balance, suggesting that heterogeneous regulation of local endothelial cell activity underlies the variable thrombotic potential in different vascular beds. The level of expression of the pathways mediating local hemostatic regulation may be critical for the prothrombotic predisposition of individual vascular beds.

The integration of the results of genetic murine studies with clinical and biochemical research provides a unique insight into the role of endothelial heterogenecity in a local hemostasis regulation. The heterogeneous expression of genes may reflect the local adaptation of ECs to perform different functions in specific tissues or may result from differences in local environmental conditions (e.g., blood flow and pressure, and oxygen level) that alter the gene expression pattern.

Several mechanisms are responsible for particular local endothelium features. First, extracellular signals including growth factors, cytokines, mechanical forces, coagulation factors, components of extracellular matrix, neighboring cells and circulating lipoproteins take place [7,8]. Excellent example of such functional and structural complementarity is the brain neurovascular unit. Second mechanism is signaling pathway specific to endothelial cell subtype. ECs from various vascular beds response differentially to the same signals, e.g. the production of PGI_2 is decreased in ECs from cerebral vessels but not from the coronary artery after stimulation with plasma from patients with thrombotic thrombocytopenic purpura [9]. Further example is the LPS-TNF-α mediated downregulation of murine vWF gene expression in large and small vessels of brain and lung, while upregulation in heart and kidney was observed [10]. Finally, at the level of transcription, e.g. in transgenic mice a short 733 bp region of the vWF promoter directed expression was found only in ECs of the brain, whereas a promoter that contained additional DNA elements directed gene expression in ECs of the heart and skeletal muscle [7].

The approval of the concept of vascular/organ heterogenecity of hemostasis leads to better understanding of pathogenesis of thrombotic and bleeding disorders and may at least partially explain why not all the patients suffering from myocardial infarction do not develop a stroke. Taken together, these results suggest that vascular ECs may influence hemostasis in a highly tissue-specific manner.

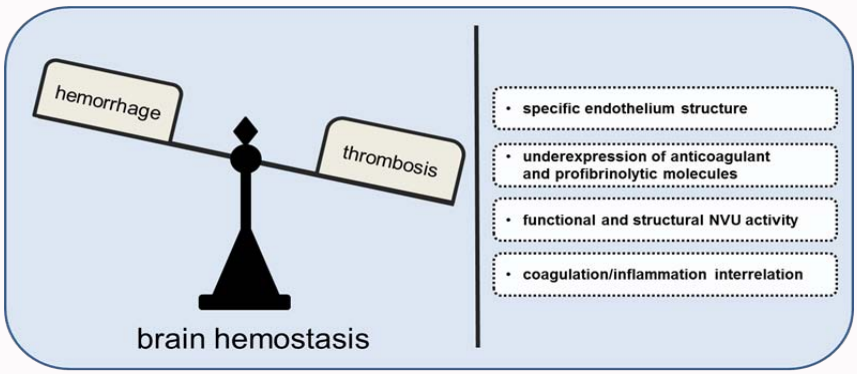

Figure 2: Major factors that determine the brain prothrombotic potential.

Brain Vascular Endothelium Prothrombotic Potential

In the brain physiological hemostatic potential is considered to be considerably high [11,12]. The brain microvasculature presents an unusually consistent configuration of structural and functional organization that offers remarkably degree of protection against hemorrhage [13]. The presence of tight endothelial junctions in systemic capillaries causes structural protection against blood loss. These tight junctions are widely present in brain capillaries of the blood-brain barrier (BBB). Brain vascular endothelium is characterized by low expression of variety of anticoagulant and profibrynolytic factors.

TF is a transmembrane glycoprotein that is expressed in extravascular tissue, particularly in fibroblasts and vessel smooth muscle cells, serving as a hemostatic "envelope" ready to activate coagulation upon vascular injury. It is not detectable in normal intact endothelium. ECs and adhered leukocytes may express active TF as a response to injury or to inflammatory stimuli such as endotoxins or cytokines. TF expression is high in brain tissue both in the capillaries and others components of BBB [14,15]. Under normal conditions, TFPI is expressed primarily by microvascular endothelium though TFPI expression in the brain is very low [15,16]. On the other side, large differences in FVIII gene expression among different murine tissues was observed. FVIII mRNA transcripts were highly enriched in hepatic endothelial cells, with intermediate enrichment in kidney endothelial cells and little to no enrichment in the endothelial cells of heart or brain in mouse [17]. vWF plays a substantial role in local platelet-arterial wall interactions. High expression of vWF in ECs of the brain arteries and microvessels may also contribute to the protection mechanism against local vascular injury in the brain [10].

Activated protein C (APC) is a normal plasma component that provides systemic antithrombotic control. It is one of the important antithrombotic enzyme which acts by inhibiting activated clotting factors Va and VIIIa [18]. APC is generated at the endothelial surface when TM binds thrombin and terminates its prothrombotic actions, forming the TM-thrombin complex that activates protein C. Although, TM is abundant in most systemic vasculatures, immunohistochemical studies revealed very limited TM expression in the human cerebrovasculature [19-21]. Supplementary evidence arises from a variety of knockout mice with TM deficiency. Mice with heterozygous TM deficiency and with modified TM containing a single amino acid substitution (producing vastly reduced ability to activate protein C) showed extensive fibrin deposition in heart, while brain did not demonstrate such phenomenon [22]. This implies tissue specific regulation of the platelet activation, coagulation pathways and fibrinolysis. The effect of the murine TM gene mutation (single amino acid substitution) was further studied using endotoxin, which provoked fibrin deposition in the lung and kidney; however, the brain was again spared [23]. The importance of the protein C antithrombotic mechanism in brain has not yet been completely established, and it is unknown whether functional TM is present in sufficient quantity to generate APC during cerebrovascular insufficiency. However, weakened protein C antithrombotic mechanism in brain was observed in patients with acute ischemic stroke [24]. Moreover, a modified APC, 3K3A-APC which has reduced anticoagulant activity and full cytoprotective properties is now extensively evaluated in preclinical and I phase clinical studies as new drug for ischemic stroke patients [25,26].

Numerous data suggest an important role of ECs for the brain regulation of fibrinolysis. *In vitro* and *in vivo* studies demonstrated t-PA and PAI-1 appearance in different sizes of microvessels including brain capillaries [27-29]. A large pool of free PAI-1 in brain microvessels was shown [30]. There is also limited t-PA expression by brain endothelium compared to the peripheral vasculature [27,31]. It was also shown that reduced expression of t-PA in brain capillaries is associated with increased infarct size following transient middle cerebral artery occlusion (tMCAO) in diabetic and nicotine stroke models [32-34].

It was demonstrated that mice with combined t-PA and u-PA deficiency to provoke thrombosis, presented extensive fibrin deposition in liver, lung, intestine, and uterus while thrombosis in brain vessels was not reported even after endotoxin injection [35]. Further studies confirmed 10- to 20-fold increase of fibrin deposition in vessels of lung, spleen, heart, and liver but not in brain in t-PA and u-PA deficient mice [22].

The above data support the suspicion that neither APC nor t-PA/u-PA-dependent pathways are necessarily involved in fibrin deposition in the blood vessels of the brain.

Neurovascular Units as a Regulator of Brain Hemostasis

Neurovascular unit (NVU) is the fundamental structural and functional element in the central nervous system (CNS). NVU is composed of ECs, pericytes, basal lamina, astrocytes, microglia and neurons [36]. The growing amount of data indicates the role of NVU in brain-specific hemostasis regulation (Figure 1). It becomes also

clear that effects of recanalization of stroke outcomes are influenced by the functions of different components of the NVU.

Pericytes are a group of contractile cells that are located between ECs, astrocytes, and neurons and almost entirely embedded within the basal lamina. Brain pericytes have functional properties consisting of contraction, mediation of inflammation, and regulation of endothelial cell activity [37]. Pericyte-endothelial coculture studies have shown the capacity of pericytes to induce and associate rapidly with capillary-like structures, as well as activating ECs and upregulating integrins [38]. Pericytes offer additional hemorrhage protection creating a structural barrier opposite to capillary tight junctions, preventing red blood cells exit [39].

It is well documented that brain pericytes play an important role in regulating brain endothelial fibrinolysis. They decrease t-PA mRNA and protein expression in ECs. Furthermore, pericytes amplify lipopolysacharide (LPS)-induced enhancement of endothelial PAI-1 [40]. The pericyte-dependent effects occur even after they are removed from ECs and incubated with LPS. These findings indicate a conditioning effect of pericytes on ECs by soluble factors and suggest a unique, brain-specific hemostasis response to an inflammatory stimulus. Such a response may contribute to a thrombotic preference within the brain vasculature during inflammatory challenge. In fact, this impaired fibrinolysis (decrease of t-PA and increase of PAI-1) has important implications, since an increased risk of brain infarction in patients with precedent infection/inflammation was observed [41].

Furthermore, pericytes can activate and propagate the coagulant response trough the extrinsic pathway. They have ability to express TF and they provide a membrane surface for assembly of prothrombinase complex [42]. On the other hand, molecule protease nexin-1 (PN-1) with anticoagulant activity is expressed in brain pericytes. PN-1 is a member of the serpin superfamily and has inhibitory activity to serine proteases [43]. Under physiological conditions, PN-1 is primarily an inhibitor of thrombin [43]. Free PN-1 is bound to proteins of the extracellular matrix, and its binding to heparin-like glucosaminoglycans increases significantly its interaction with thrombin. The importance of PN-1 expression in brain may be related to the low level of brain TM. The TM-thrombin complex activates protein C, thus reversing the procoagulant effects of thrombin. Pericyte-dependent PN-1 might have compensatory role for under expression of other endogenous anticoagulants in the brain.

Previous results have shown that pericytes are contractile cells, which may control blood flow in CNS microvessels [44,45]. According to the energy requirements of nervous tissue they may contract or dilate in physiological conditions [46]. Recently, it was demonstrated that pericyte dilatation is mediated mainly in NO-dependent manner [47]. Moreover, some markers of increased energy utilization, including lactates, adenosine, low pH as well as short time exposure (10 min) to reactive oxygen species (ROS) can also relax pericytes, while long time exposure (30 min) to ROS leads to pericyte contraction [39]. These close anatomical and functional interactions between pericytes and other NVU components play pivotal role in the progression of stroke pathology. Brain microvascular pericytes contract one hour after artery blockage and remain contracted despite reopening of the occluded artery. It was shown that pericyte contraction during ischemia resulted from oxidative and nitric stresses. Pericyte contraction is reduced when oxidative and nitric stress mechanisms are inhibited, and improved microvascular flow promotes cerebral tissue survival [48]. It was observed that constricted pericytes die after cerebral ischemia and such death was not reduced by free radical scavenging, and contributed to ongoing neuronal damage. This may at least partially explain why treatment of ischemic stress with oxidative and nitric stress inhibitors has been often unsuccessful [45,49]. It could be suggested that approaches targeting pericyte responses after ischemia and reperfusion may provide new therapies for ischemic stroke (reviewed elsewhere, [50].

It is well documented that brain pericytes play both immunoactive and immunosuppressive roles. In a pericyte-deficient mouse model, the upregulation of intercellular adhesion molecule-1 (ICAM-1) and large leukocyte infiltration was observed [37,51]. This indicates that pericytes can prevent leukocyte infiltration into the brain. On the other hand, the pro-inflammatory properties of pericytes are shown by continuous expression of adhesion molecules (e.g. ICAM-1 and VCAM-1) [52] and are associated with leukocyte recruitment [53]. Moreover, under physiological conditions pericytes secrete interleukines (e.g. IL-9, IL-10), tumor necrosis factor α (TNF-α), and interferon gamma (IFN-γ). During immune reaction pericytes produce great amounts of inflammatory cytokines, ROS and NO [53,54]. They are also involved in regulating peripheral immune cell infiltration in response to neurovascular injury [46].

Substantial data support a role for astrocytes in brain-specific hemostasis regulation. It was shown that the components of fibrinolytic system: t-PA, u-PA, plasmin and PAI-1 are expressed in astrocytes [55,56]. The role of astrocytes in controlling coagulation in the brain was also confirmed. It was shown that astrocytes are the main source of TF in CNS and its expression is related to microvessel density within brain [12,57]. Moreover, TF expression was enhanced in endotoxemia in brain of LPS-subjected rabbits [58]. On the other hand, the expression of PN-1 – a potent thrombin inhibitor, has been shown in astrocytes *in vivo* [59]. It was also demonstrated that PN-1 also inhibits u-PA and plasmin [59]. Under pathologic conditions e.g. Alzheimer`s disease, a reduction of perivascular PN-1 expression was observed [60]. The significance of this interaction in terms of hemostatic balance is not entirely clear. Furthermore, it was shown that the kunitz protease inhibitor domain of protein nexin-2 (PN2) inhibited factor XIa leading to the reduced volume and fraction of ischemic brain tissue in the MCAO model in mice [61].

Protease-activated receptor 1 (PAR1) is frequently expressed in brain astrocytes [62]. PAR1 is activated by serine proteases in the bloodstream (which take a part in signaling cascades involved in the formation of blood clots). Under physiological conditions, the BBB prevents these proteins from spreading out of the lumen of vessels. However, during cerebrovascular injury thrombin and plasmin mediate intracellular signaling via PAR1 and PAR2 receptors in the brain neuropil which is one of the integrating points between the coagulation pathway and the inflammatory response [63].

Moreover, astrocytes also regulate thrombotic potential of ECs. They control brain microvascular endothelial fibrinolysis (negatively regulate human brain capillary endothelial t-PA, while augmenting PAI-1) as well as TM expression via transforming growth factor-beta (TGF-β) [64,65]. It was shown that astrocyte-endothelial coculture produces even a 20-fold downregulation of TM compared with monocultures [66].

Another factor involved in astrocyte-dependent hemostasis regulation is PGI_2. PGI_2 synthesis and release was found in primary cultures of rat astrocytes [67] and further studies showed PGI_2

synthesis in hydrogen peroxide (H_2O_2)-stimulated astrocytes. The specific function of PGI_2 in relationship to hemostasis in physiology and pathology of the brain still remains to be established [68,69].

Summing up, these findings suggest an important and complex role for pericytes and astrocytes coordinating hemostasis within the microvasculature with net antifibrinolytic and procoagulative effects. Furthermore, these observations support the concept that NVU components may play an important role in regulating coagulation events after cerebrovascular injury.

Contact-Kinin Pathway in Brain Hemostasis

Considerable efforts have been made in recent years to understand the role of XII pathway in brain hemostasis. The contact-kinin pathway is initiated on activation of FXII via contact with a negatively charged cell surface or negatively charged inorganic polyphosphates released from activated platalets [70]. FXII was believed to play no role for blood clotting *in vivo* based on the fact that hereditary FXII deficiency in humans does not increase the risk of spontaneous or injury-related bleeding [71]. This view has been challenged by the generation of $FXII^{-/-}$ mice [72]. Like humans, these animals display normal bleeding time and no signs of spontaneous hemorrhage. However, the ability to form occlusive thrombi was severely impaired in aorta, carotid artery and mesenteric vessels. Moreover, thrombus was unstable and fragmented thus more prone to lysis and washing out by the blood stream [72].

These data giving rise to the intriguing perspective of targeting thrombus formation without inducing bleeding complications in thromboembolic diseases e.g. ischemic stroke. In fact $FXII^{-/-}$ mice were largely protected from cerebral ischemia [73]. Twenty-four hours after tMCAO, stroke size in $FXII^{-/-}$ mice was reduced and better functional outcome was found. This protection was sustained over time. Intravenous infusions with human FXII fully restored the predisposition for ischemic brain damage in $FXII^{-/-}$ mice, indicating that the protective effect was specifically related to the absence of FXII. Most notably, ischemic brain challenge by tMCAO did not increase the risk of intracranial bleeding in $FXII^{-/-}$ mice. Corresponding observations were obtained upon pharmacological inhibition of FXII by selective inhibitor rHA-Infestin4 in murine stroke model [74].

These observations were verified by the study showing improved stroke outcomes and reduced thrombosis in mice after blocking FXI, the primary substrate of activated FXII and FIX which is located downstream of FXII and FXI [73,75]. It was demonstrated however, that FIX deficiency is associated with a substantial bleeding phenotype since FIX is also activated by the FVIIa-TF complex, which initiates the extrinsic pathway of coagulation. These studies clearly pointed out the central pathophysiological role of the intrinsic coagulation pathway in brain ischemia/reperfusion injury.

To date no controlled clinical trial has correlated contact system deficiency with thrombotic disease, since the deficiency of contact system is rare in humans [76]. Furthermore, relationships of FXII polymorphisms with thrombotic outcomes have been conflicting [77]. However, the deficiency of FXI protects from stroke in Ashkenazi Jewish group [78]. It is worth to underline that the incidence of stroke, but not myocardial infarction, was significantly lower in population with severe FXI deficiency [79]. Moreover, FXI levels are higher in patients with stroke and are associated with poor prognosis [80].

The contact-kinin pathway plays an important role in the thromboinflammatory pathology of ischemic stroke not only by fostering vascular permeability and inflammation via kinins such as bradykinin, but also by promoting thrombus formation through activation of the intrinsic pathway [81,82]. FXIIa cleaves plasma prekallikrein to form plasma kallikrein, which in turn cleaves high molecular weight kininogen (HMWK), inducing the release of the proiflammatory hormone bradykinin from HMWK. Plasma kallikrein by a positive feedback loop can also activate FXII. The involvement of this system in experimental stroke was demonstrated as both genetic and pharmacological inhibition of plasma kallikrein protected mice from ischemic stroke without an increase in infarct-associated hemorrhage [83]. Plasma kallikrein deficiency led to reduced intracerebral thrombosis, enhanced cerebral blood flow and reduced local inflammation. Also HMWK was documented as key mediator of experimental ischemic stroke by enhancing microvascular thrombosis and inflammation then its absence has been shown to be protective after tMCAO in mice [84]. Thus, by targeting both coagulation and inflammation, plasma kallikrein inhibition may offer a safe therapeutic strategy for ischemic stroke.

The presumption that FXII links thrombotic activity with inflammation was supported by studies in bradykinin receptor 1 (B1R) deficient mice, in which the local inflammatory response in the brain was reduced while infarct size was smaller. Also blockade of B1R protects against ischemic brain damage and is associated with less edema formation and attenuation of the local postischemic inflammatory response in murine model of brain infarction [85].

A growing evidence supports that C1-inhibitor exhibits anti-inflammatory and antithrombotic mode of action blocking the contact-kinin system by inhibiting FXIIa and plasma kallikrein. Recently, it was demonstrated that C1-inhibitor improved stroke outcome by interfering with main mechanisms of ischemic brain damage like thrombosis and inflammation in mouse and rat models of brain ischemia [86]. In support of the experimental data, the contact-kinin pathway has been shown to be activated in patients with stroke [87]. Thus, targeting the FXIIa-driven contact system seems to be a promising and safe target for stroke therapy with additional anti-inflammatory effects.

Protease Nexin-2/Amyloid – β-Protein Precursor as a Cerebral Anticoagulant

The amyloid β-protein precursor (AβPP) is the parent molecule to the amyloid β-protein (Aβ) that accumulates in the brains of patients with Alzheimer disease and related disorders [88]. Cerebral PN-2/AβPP levels are also increased with age after brain injury. Secreted isoforms of AβPP that contain the kunitz proteinase inhibitor domain are analogous to the previously identified cell-secreted PN-2. PN-2/AβPP is a potent inhibitor of several key prothrombotic proteinases e.g., intrinsic blood coagulation factor XIa [89]. PN-2/AβPP is also abundantly expressed in circulating blood platelets.

Recently, the murine genetic studies have significantly contributed to understanding of the role of AβPP in brain hemostasis regulation. Deletion of PN-2/AβPP in AβPP gene knockout mice resulted in a significant increase in the carotid artery thrombosis. Similarly, platelet PN-2/AβPP transgenic mice developed larger hematomas in experimental intracerebral hemorrhage, whereas AβPP gene knockout mice exhibited reduced hemorrhage size. In contrast, in transgenic mice that modestly over express PN-2/AβPP in brain, severity of hemorrhage in model of experimental intracerebral

hemorrhage was increased. These findings indicate that PN-2/AβPP has a significant role in regulating cerebral thrombosis and that even modest increase of this protein can enhance cerebral hemorrhage [90]. The animal data are consistent with age- and disease-related increases of this protein in brain that may contribute to the severity of hemorrhage. Cerebral amyloid angiopathy is an important cause of spontaneous intracerebral hemorrhage in the elderly [88,91].

Summary

It was previously thought that antithrombotic and fibrinolytic pathways are involved in the brain hemostasis to a smaller degree as compared with other organs, which implies that protection against hemorrhage is a higher priority for this organ. Prothrombotic background of the brain may provide evolutionary advantage protecting the brain from neonatal intracranial hemorrhage. However, nowadays with development (with age) cardiovascular risk factors, this prothrombotic setting may predispose to brain infarction (Figure 2). Future genomic research in conjunction with further *in vitro* and *in vivo* experimentation, and clinical studies will provide more comprehensive understanding of the local hemostatic mechanisms responsible for stroke pathology and may provide a new approach for specific prediction, prevention and treatment of thrombotic brain damage. Directions to novel drugs development intended as a site directed therapy are particularly compelling neuropil (area in the nervous system composed of mostly axons, dendrites and glial cell processes that forms a synaptically dense region containing a relatively low number of cell bodies).

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