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The influence of the amyloid β-protein and its precursor in modulating cerebral hemostasis

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Abstract

Ischemic and hemorrhagic strokes are a significant cause of brain injury leading to vascular cognitive impairment and dementia (VCID). These deleterious events largely result from disruption of cerebral hemostasis, a well-controlled and delicate balance between thrombotic and fibrinolytic pathways in cerebral blood vessels and surrounding brain tissue. Ischemia and hemorrhage are both commonly associated with cerebrovascular deposition of amyloid β-protein (Aβ). In this regard, Aβ directly and indirectly modulates cerebral thrombosis and fibrinolysis. Further, major isoforms of the Aβ precursor protein (AβPP) function as a potent inhibitor of pro-thrombotic proteinases. The purpose of this review article is to summarize recent research on how cerebral vascular Aβ and AβPP influence cerebral hemostasis.

Keywords

Ischemia; Hemorrhage; Cerebral hemostasis; Amyloid β-protein; Cerebral amyloid angiopathy; Amyloid β-protein precursor; Proteinase inhibition

1. Introduction

Vascular cognitive impairment & dementia (VCID) is defined as a form of dementia that is triggered by damage to cerebral blood vessels or cerebrovascular disease. There are many types of cerebral vascular abnormalities that can cause VCID including hypertension, arteriosclerosis, diabetes, cerebral amyloid angiopathy (CAA), as well as ischemic and hemorrhagic stroke [1,2]. Ischemic and hemorrhagic stroke result from disruption of cerebral hemostasis, a delicate balance between structural and functional pro- and anti-coagulant and fibrinolytic mechanisms that strives to maintain vascular integrity and blood flow [3]. For example, the pro-coagulant cascade is initiated immediately after vascular injury involving both cellular and circulating components leading to the formation of a fibrin clot sealing the damaged vessel. On the other hand, anti-coagulant mechanisms secure the precise control of coagulation both in scope and location to secure the flow of blood.

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Cerebral hemostasis is of utmost importance since an imbalance between the coagulant and fibrinolytic systems in the brain can lead to one or both forms of stroke. Stroke is a common cause of death and disability throughout the world. In the United States alone nearly 800,000 people suffer a new or recurrent stroke each year, and approximately 140,000 will die from stroke, making this cerebrovascular disease the fifth leading cause of death [4,5]. It is estimated that annual cost of care for stroke patients exceeds $40 billion dollars in United States [4,5]. These points underscore the importance of understanding the factors that participate in the maintenance of cerebral hemostasis under normal conditions and in pathological events during cerebral vascular injury.

Approximately 85% of the strokes that occur annually in the United States are ischemic in nature. Ischemic strokes are largely caused by either local thrombosis or embolism leading to interruption of the blood supply to the brain resulting in tissue hypoperfusion, hypoxia, the death of brain tissue and focal neurological deficits [6]. Thrombotic ischemic stroke occurs when there is aberrant activation of the coagulation cascade concurrent with aggregation and activation of blood platelets at the site of cerebral vascular injury leading to formation of a platelet/fibrin rich clot that occludes the vessel and prevents normal blood flow. In addition, congenital or acquired abnormalities in platelets and coagulation inhibition resulting in a hypercoagulable state, although poorly defined, may also contribute to thrombotic ischemic stroke [7,8].

The remaining 15% or so of strokes are hemorrhagic in origin and result largely from rupture of meningeal or small penetrating cerebral arteries leading to subarachnoid or intracerebral hemorrhage, respectively [9,10]. Although less common, hemorrhagic strokes are associated with a higher mortality rate than ischemic stroke [9–11]. During cerebral hemorrhage primary tissue damage occurs at the time of hematoma formation when blood initially spills into the brain parenchyma [12]. Secondary tissue damage and edema likely result also from blood products including activated coagulation factors and thrombin leading to neural injury and cell death [13,14]. Although rare, spontaneous cerebral hemorrhage may also result due to specific disorders involving coagulation factors and platelets [15,16].

Although ischemic and hemorrhagic strokes may originate via different mechanisms and initially manifest in distinct manners (i.e. vessel occlusion vs. vessel rupture and bleeding), they are not mutually exclusive. For example, primary ischemic stroke can lead to secondary hemorrhagic conversion particularly after reperfusion [17,18]. Alternatively, some studies have suggested that primary hemorrhagic stroke can lead to secondary hypoperfusion and ischemia downstream from the site of vessel rupture [19,20]. Therefore, both types of stroke may be interrelated and have similar events associated with them that disrupt hemostasis.

The above points concerning ischemic and hemorrhagic stroke, both of which are highly deleterious to brain survival and function, underscore the importance of maintaining proper cerebral hemostasis. Here below, is presented a summary of what is known about how the amyloid β-protein (Aβ) and its precursor protein (AβPP), two abundant factors in the CNS, can influence cerebral hemostasis and, under disease conditions, may result in potentially harmful consequences.

2.1 Amyloid β-Protein

The amyloid β-protein (Aβ) is 39–43 amino acid peptide that is best known as the chief component of senile plaques that are present in the brain parenchyma of patients afflicted with Alzheimer's disease (AD) and related disorders [21–23]. Aβ is a proteolytic product of the amyloid β-protein precursor (AβPP), which is a type I integral membrane protein encoded by a gene located on chromosome 21 [24–26]. Full-length AβPP can undergo proteolytic cleavage by an aspartyl proteinase, termed β-secretase, at the amino terminus of the Aβ domain [27,28]. Subsequent cleavage of the remaining amyloidogenic membrane spanning AβPP carboxyl terminal fragment by the presenilin γ-secretase complex liberates the primarily 40 or 42 residue Aβ peptide [29–31]. Soluble Aβ peptides are normal biological products that can be readily detected in interstitial and cerebrospinal fluids in the brain as well as in plasma [38,39]. Alternative to the amyloidogenic processing, full-length AβPP can undergo cleavage by α-secretase at the carboxyl terminal side of Lys^{16} of the Aβ domain. This predominant cleavage event generates a non-amyloidogenic membrane spanning carboxyl terminal fragment and truncated secretory forms of AβPP that are released into the extracellular environment and have a number of potential biological functions as will be described below [34,35].

2.2 Cerebral amyloid angiopathy

In addition to parenchymal plaques another key site of pathological Aβ deposition is within and along the walls of cerebral blood vessels and capillaries, a condition known as cerebral amyloid angiopathy (CAA) [36–38]. Not surprisingly, with the involvement of Aβ CAA is the most common vascular comorbidity found in the brains of AD patients [36–38]. In addition to the prominent CAA that is present in AD and in spontaneous cases of this condition, several monogenic, familial forms of CAA exist that result from specific mutations that reside primarily within the middle region of the Aβ peptide sequence of AβPP gene [39–44] (Fig. 1). The most recognized example of familial CAA is the Dutch-type E22Q substitution in Aβ that causes early and severe cerebral vascular amyloid deposition [39,40]. Pathologically, this disorder is characterized by extensive fibrillar Aβ deposition that occurs in the cerebral blood vessels, but with an absence of parenchymal fibrillar amyloid plaques that are a key feature of AD [45–47]. Typically, patients develop recurrent, and often fatal, intracerebral hemorrhage at mid-life [45,46,48] Clinically, Dutch-type CAA is accompanied by progressive cognitive impairment that appears to be driven by the extensive vascular amyloid deposition present in this disorder [49–51].

Individuals afflicted with the Flemish-type A21G, Italian-type E22K and Piedmont-type L34V familial CAA are also prone to develop recurrent hemorrhagic strokes, cognitive decline and dementia [41,42,44]. In contrast, hemorrhages are more rare in the Arctic-type E22G disorder although this form is accompanied by enhanced Aβ protofibril formation and abundant parenchymal amyloid pathology [52,53]. Hemorrhages are also less common in the Iowa-type D23N form of familial CAA although present in some kindreds [54,55]. However, similar to the Dutch-type CAA disorder, Iowa-type CAA is pathologically characterized by early and severe cerebral vascular amyloid deposition, again with the noticeable absence of parenchymal fibrillar plaques [56]. Clinically, Iowa-type CAA is also

associated with a progressive cognitive impairment that again appears to be promoted by the extensive microvascular amyloid deposition [54]. Thus, these familial CAA mutations target Aβ deposition to the cerebral vasculature where it has profound consequences that impact cerebral vascular function and integrity contributing to cognitive decline and dementia.

The reason as to why familial CAA mutant forms of Aβ lead to preferential accumulation of fibrillar amyloid in the cerebral vasculature while the brain parenchyma is largely spared of fibrillar amyloid deposits is unclear. However, several findings concerning CAA mutant Aβ peptides may underlie their predilection for vascular deposition. First, the familial CAA mutations tend to cluster around positions E22 and D23 in Aβ (Fig. 1). For example, the Dutch-type (E22Q) and Iowa-type (D23N) mutations are adjacent within the Aβ peptide and both result in loss of a negative charge at their respective sites. Previous *in vitro* studies have shown that both of these mutations increase the fibrillogenic and cerebrovascular cell pathogenic properties of Aβ compared with wild-type Aβ [57–62]. Second, previous studies showed that GM3 ganglioside, which is abundantly found in cultured cerebrovascular cells and in isolated cerebral vessels, selectively promotes fibrillar assembly of CAA mutant forms of Aβ [63,64]. Third, CAA mutant forms of Aβ exhibit drastically reduced clearance from brain via cerebrospinal fluid and across the blood-brain barrier into the peripheral circulatory system [65,66]. This deficiency in clearance could lead to the accumulation of CAA mutant Aβ peptides at the cerebral vessels. Finally, the presence of CAA mutations in Aβ may induce conformational changes that preferentially target them for assembly and deposition in the cerebral vasculature. For example, it was shown *in vitro* experiments that the Iowa CAA mutant form of Aβ can adopt novel β-sheet structures and assemble into fibrils with a unique anti-parallel geometry, in contrast to the preferential parallel, in-register fibril configuration commonly observed with non-mutated wild-type Aβ peptides [67,68]. Together, a combination of these altered functional and structural properties related to CAA mutant forms of Aβ likely contribute to their selective and robust accumulation as fibrillar deposits in the cerebral vasculature.

2.3 Ischemic consequences of cerebral vascular Aβ

Cerebral vascular Aβ can contribute to cerebral vessel dysfunction and injury resulting in reduced cerebral blood flow, hypoperfusion and ischemia through several pathogenic mechanisms. First, it has been demonstrated that soluble Aβ possesses vasoconstrictive properties that can reduce cerebral blood flow, cause vascular uncoupling and render the brain more susceptible to ischemic injury [61–64]. These effects can be facilitated by luminal and abluminal soluble Aβ and appear to be mediated through its ability to enhance production of free radicals and pro-inflammatory pathways in cerebral vascular cells [62,63,65–67]. Second, the deposition of amyloid in cerebral blood vessels causes degeneration of cerebrovascular smooth muscle cells, microvascular pericytes and perhaps microvascular endothelial cells leading to loss of vascular function in regulating cerebral blood flow [61,68–71]. Also, the accumulation of cerebral vascular amyloid can lead to thickening of the cerebral blood vessel walls resulting in restricted cerebral blood flow and ischemic infarcts [72–74]. In response to amyloid vascular activation can occur in cerebral vascular cells resulting in increased expression of thrombin that is directly neurotoxic [75,76]. Also, thrombin can promote fibrin clot formation and vessel occlusion further

leading to reduced cerebral blood flow and ischemia. Finally, there is abundant experimental evidence that Aβ can promote platelet activation and support platelet adhesion and aggregation [77–80]. The consequences of this can enhance thrombus formation leading to perturbed cerebral blood flow and ischemic infarcts [79,80]. In any case, neuroradiological findings have revealed a strong correlation between CAA and cortical infarcts, white matter hyperintensities and microstructural tissue abnormalities all contributing to cognitive decline [81–84].

2.4 Hemorrhagic consequences of cerebral vascular Aβ

Alternatively, the degeneration and loss of cerebral vascular cells, a common consequence of CAA, can cause decreased contractile properties of the vessel and promote loss of vessel wall integrity and cerebral hemorrhage [85–87]. Additionally, Aβ can impact several proteolytic systems in the cerebral vasculature in ways that can promote hemorrhage. First, regarding the thrombosis pathway cerebral vascular amyloid can inhibit coagulation by binding the fibrin crosslinker Factor XIIIa thus potentially interfering with normal fibrin clot formation [88]. Also, degenerating smooth muscle cells in amyloid laden cerebral vessels have been implicated in the over production of AβPP, an inhibitor of pro-thrombotic enzymes (as discussed in detail below) [47,68,69]. Of note, cerebral vascular amyloid can also bind AβPP and stimulate its anti-thrombotic activity [47,89]. Thus, in the presence of CAA inhibiting normal thrombosis and fibrin clot formation would create an environment conducive to bleeding.

Second, Aβ can increase the expression and activation of certain matrix metalloproteinases (MMPs), a large family of neutral, Zn^{2+}-containing proteinases that degrade a wide array of extracellular matrix components and play a pivotal role in ischemic and hemorrhagic stroke [90,91]. In particular, MMP-2 and MMP-9 have been implicated in the deterioration of blood-brain barrier integrity under certain pathological conditions. For example, evidence from both animal models and human studies support a role for MMP-9 in disruption of the blood-brain barrier during stroke and neuroinflammatory conditions [92–94]. Direct intracerebral injection of MMP-2 has been shown to cause opening of the blood-brain barrier and causes intracerebral hemorrhage by disrupting the ECM [95,96]. Studies have shown that in response to Aβ cerebral vascular smooth muscle cells and endothelial cells increase their expression and activation of MMP-2 and MMP-9 [97–100] and in CAA-related hemorrhage patients [101,102]. These findings suggest that specific MMP expression and activation in cerebrovascular cells in response to pathogenic amyloid deposition may contribute to loss of vessel wall integrity and hemorrhaging in CAA.

Lastly, in response to Aβ cerebral vascular cells increase the expression and activation of plasminogen activators [103,104]. As discussed in more detail below, the potential disruption of fibrinolytic pathways by Aβ is complex but, in certain situations, can create an environment prone to loss of vessel wall integrity and hemorrhage. In any case, clinical neuroimaging studies in patients have shown that CAA can indeed promote lobar cerebral microbleeds, cortical superficial siderosis and intracranial hemorrhage, all deleterious processes that can contribute to VCID [105–107].

2.5 Aβ interactions with the fibrinolytic system

Tissue-type plasminogen activator (tPA) has been used clinically as an effective thrombolytic agent for the treatment of ischemic stroke [108]. However, a serious side effect of tPA is a substantially increased risk for developing intracerebral bleeding [109,110]. Further, it is increasingly recognized that tPA-induced hemorrhage is associated with the presence of CAA [111,112]. This phenomenon may be explained by previous studies showing that Aβ and fibrin deposits, both, β-sheet containing fibrillar aggregates, share common structure and antibody epitopes [113,114]. In fact, like fibrin, fibrillar Aβ can bind tPA and stimulate its activity to enhance plasminogen activation [115–117]. Thus, these fibrin-mimicry activities of fibrillar Aβ might underlie the prevalence of tPA-induced hemorrhage at sites of CAA as observed in patients and in experimental mouse models [109,110,118].

On the other hand, Aβ may have other deleterious effects on fibrinogen and clot formation. Normally, fibrinogen is restricted to the blood and is excluded from the brain parenchyma due to the presence of the blood-brain barrier. However, it has been reported that fibrin[ogen] is co-localized with CAA deposits and in the brain parenchyma in AD patients and in mouse models of cerebral amyloid deposition [119–121]. Further, the fibrin accumulation in CAA enhances cerebral vascular dysfunction and damage [119]. Subsequently, it has been shown that Aβ peptides can interfere with normal fibrin polymerization resulting in denser clots that are resistant to proteolytic degradation [122,123]. This interaction appears to involve Aβ binding to the C-terminus of the fibrinogen β-chain and disruption of this interaction with a small molecule inhibitor restored normal fibrin clot formation [123,124]. Thus, the impact of Aβ on fibrin clot formation and clot dissolution is complex and suggests that the pathological activities of Aβ in this regard are likely dependent on the environment and circumstances of the relevant event.

3.1. Cerebral vascular proteinase inhibitory functions of AβPP

The above sections reviewed the effects of Aβ on cerebral vascular functions and pathology that can contribute to ischemic and hemorrhagic stroke. The following sections will summarize the cerebral vascular effects of its precursor AβPP. As mentioned above, AβPP is most recognized as the parent molecule of the Aβ peptide. Full length AβPP is translated from primarily three alternatively spliced mRNAs resulting in polypeptides of 695, 751 and 770 amino acids: the latter two species contain an additional Kunitz-type serine proteinase inhibitor (KPI) domain [125–127]. Whereas the 695 isoform of AβPP that lacks the KPI domain is primarily expressed by neuronal cells in brain, the KPI domain-containing 751 and 770 isoforms are also abundant in brain, primarily expressed by glia cells, but are also expressed in a variety of non-neural tissues most notably circulating blood platelets [128–131].

In addition to serving as the precursor to the Aβ peptides a number of biologically active domains have been identified in the N-terminal regions of secreted AβPP molecules upstream of the Aβ domain. For example, two high affinity binding sites for heparin have been identified on regions of AβPP encoded by exons 3 and exon 9 that may participate in

substrate adhesion or mediate AβPP dimerization [132,133]. A high affinity Zn^{2+} binding domain on AβPP, located between the cysteine-rich and negatively charged regions of the protein, was shown to potentiate AβPP binding heparin [134]. Further regarding metal interactions, a Cu^{2+} binding site was identified on AβPP between residues 135–155 that can reduce copper and may enhance production of hydroxyl radicals [135,136]. A high affinity fibrillar Aβ binding domain was also identified between residues 95–118 in the amino terminal region of AβPP [137,138]. Additional domains on the extracellular portion of AβPP have been implicated in cell adhesion [139,140], growth promoting activity [141,142], and regulation of Ca^{2+} homeostasis [143,144].

Another significant functional region on the extracellular portion of the 751 and 770 isoforms of AβPP is the Kunitz proteinase inhibitor (KPI) domain [125–127]. In fact, the secreted KPI domain-containing isoforms of AβPP are analogous to the cell-secreted proteinase inhibitor known as protease nexin-2 (PN2), which was first purified and characterized in 1987, prior to the known existence of AβPP [145,146]. PN2/AβPP was first identified to form SDS-stable complexes with the serine proteinase epidermal growth factor binding protein [147]. Subsequently, it was shown to also inhibit trypsin and chymotrypsin [145,146]. However, the most compelling findings, summarized in Table 1, were experiments showing that purified PN2/AβPP is a tight-binding inhibitor of several enzymes of the blood coagulation cascade including factors XIa, IXa, Xa, and VIIa:tissue factor with inhibition equilibrium constants in the nM to pM range [148–152].

It is noteworthy that certain ligands that bind to the amino terminal portion of the PN2/AβPP, including heparin, zinc and fibrillar Aβ were shown to further enhance its ability to inhibit factor XIa [148,153–155]. It is likely that the binding of these ligands alters the conformation of PN2/AβPP to enhance the KPI function. The potent regulation of the above coagulation factors by PN2/AβPP occurs at key points in the initiation and amplification phases leading to the conversion of prothrombin to thrombin (Fig. 2). This suggests a role for this protein in the regulation of thrombosis leading to clot formation. In support of this, both PN2/AβPP and its purified KPI domain were demonstrated to be effective inhibitors of the clotting of plasma *in vitro* [150,156].

3.2 Contribution of PN2/AβPP to the unique hemostatic environment of the brain

A role for PN2/AβPP in regulating thrombosis *in vivo* is supported by studies from several groups demonstrating that it is a very abundant platelet α granule protein that is released upon platelet activation by physiological agonists such as thrombin and collagen [128,129,149]. This indicates that platelets provide a rich circulating source of PN2/AβPP that can be delivered to sites of vascular injury upon demand and likely serves a physiological function in regulating pro-thrombotic events that occur during vascular injury [157].

The consequences of ischemic and hemorrhagic stroke, both of which are highly deleterious to brain survival and function, underscore the importance of maintaining proper cerebral hemostasis. Accordingly, the environment of the CNS appears to be distinct from other

tissues in to order to precisely control cerebral hemostasis. For example, the pro-coagulant tissue factor is most abundantly expressed in human brain compared to other tissues [158]. Tissue factor binds circulating coagulation factor VII forming a protelytically active tissue factor:VIIa complex that is essential in the extrinsic coagulation pathway leading to the generation of thrombin. On the other, the important anti-coagulant molecule thrombomodulin is expressed at extremely low levels in human brain compared to other tissues [159,160]. Thrombomodulin is an endothelial membrane protein that is an important cofactor for the activation of protein C, a proteinase which inactivates the pro-coagulant molecules factor Va and factor VIIIa. Thus, the abundance of tissue factor and paucity of thrombomodulin in brain suggests that this tissue is highly primed for thrombosis and that endogenous inhibitors of thrombosis in brain might exist. Within the brain, PN2/AβPP is expressed in the parenchyma by glial cells and in cerebral blood vessels by smooth muscle cells, microvascular pericytes and endothelial cells [68–70,161,162]. Thus, in addition to its potential systemic function regulating thrombosis via circulating blood platelets, the rich investment of KPI-containing PN2/AβPP in the central nervous system and in cerebral blood vessels suggests that this protein may also serve to function locally as an intracerebral anti-thrombotic [163]. This has particular importance for the brain as to preserve critical cerebral blood flow. In addition, studies have demonstrated the very deleterious effects of thrombin on neuronal function and viability [75,76]. Therefore, tight control of the activation of thrombin is of utmost importance in the CNS.

On the other hand, overly elevated levels or abnormal accumulation of PN2/AβPP in cerebral blood vessels my have deleterious consequences of their own. For example, in severe cases of familial CAA pathologic accumulation of KPI-containing isoforms of AβPP in the amyloid laden cerebral blood vessel walls has been reported [47]. This may result in a cerebral vessel wall possessing an unusually high anti-thrombotic potential. Such a localized cerebral hemostatic imbalance could contribute to the bleeding condition that is characteristic of familial cerebral hemorrhagic disorders and in severe cases of CAA.

3.3 Anti-thrombotic functions of PN2/AβPP: In vivo studies

As described in section 3.2 above, it has been shown that PN2/AβPP is a potent inhibitor of several key pro-thrombotic enzymes, limits the clotting of plasma *in vitro* and it richly invested in both circulating blood platelets and in the CNS, together suggesting a role for this protein in regulating cerebral thrombosis. However, to establish this requires *in vivo* studies and the use of genetically modified mice provide a means to evaluate this. To this end, studies have shown that over-expression of PN2/AβPP in transgenic mice, either in circulating blood platelets or in brain, resulted in significantly decreased cerebral thrombosis in experimental models of carotid artery thrombosis and intracerebral hemorrhage [164,165].

On the other hand, one would predict that the absence of PN2/AβPP should result in a pro-thrombotic phenotype. Deletion of genes for other thrombosis inhibitors such as anti-thrombin III or tissue factor pathway inhibitor present with a severe, and often, fatal pro-thrombotic phenotype [167,168]. However, although AβPP gene knock out (KO) mice have been previously generated, for the most part, they appear relatively normal, live normal lifespans and do not present with an overt pro-thrombotic phenotype [169]. One explanation

is that the role of endogenous PN2/AβPP in regulating thrombosis is more subtle and that under conditions that challenge AβPP KO mice with a cerebral thrombotic event would present with an observable thrombotic phenotype. Indeed, studies have shown that in an experimental model of carotid artery thrombosis AβPP KO mice exhibited a significantly reduced time to vessel occlusion [164]. Similarly, AβPP KO mice presented with significantly reduced hematoma volumes in an experimental model of intracerebral hemorrhage [164].

Although these findings confirmed that in cases of a challenged cerebral thrombotic event the absence of PN2/AβPP results in increased thrombosis, the effects were not overly robust. However, in the case of PN2/AβPP the scenario is potentially more complicated with the presence of homologous proteins with overlapping functions. Previously, the cDNAs for two proteins, AβPP-like protein-1 (APLP1) and APLP2, were isolated which share a high degree of structural homology with AβPP [170–172]. One significant difference between PN2/AβPP and APLP1 is that the latter protein does not contain a KPI domain. In contrast, the cDNA for APLP2 was shown to contain a KPI domain that is 68% homologous to the KPI domain contained in PN-2/AβPP [171,172]. It is noteworthy that similar to PN2/AβPP, mRNA for APLP2 is found in many tissues and is very abundant in brain and in platelets [171,172]. The expression, purification and biochemical characterization of the KPI domain of APLP2 was previously reported [173]. Interestingly, the pro-thrombotic proteinase inhibitory properties of the KPI domain of APLP2 and the KPI domain of PN2/AβPP revealed that they strongly overlap [173]. This suggest that the KPI domains of each protein, abundant in platelets and in brain, may have shared and compensatory activities in regulating platelet function and thrombosis during cerebral vascular injury. Indeed, the absence of APLP2 in KO mice results in a pro-thrombotic phenotype in experimental models of cerebral vascular injury and thrombosis, similar to that observed in AβPP KO mice [174,175]. Taken together, the *in vivo* studies indicate that PN2/AβPP contributes to the regulation of cerebral thrombosis and that this role may be redundantly shared with its structurally and functionally related homolog APLP2.

Conclusions

Ischemic and hemorrhagic strokes can result from imbalances in cerebral hemostatic mechanisms resulting in brain tissue injury leading to VCID. In this review I summarize what is known about how Aβ and its parent protein AβPP can contribute to cerebral vascular pathology and disruption of cerebral hemostasis. As illustrated in Fig. 3, AβPP serves as the precursor to its proteolytic product Aβ, which can prominently accumulate as fibrillar amyloid in cerebral blood vessels as CAA. Each of these entities can impact specific cellular and hemostatic processes that can either lead towards ischemia or hemorrhage. For example, reduced levels of AβPP, a potent inhibitor of the thrombosis pathway, can result in increased thrombosis and support an ischemic environment. On the other hand, elevated AβPP levels can reduce thrombosis and promote a hemorrhagic setting as well as increase production of Aβ. The presence of accumulating Aβ can progress further to assembly and deposition of cerebral vascular fibrillar amyloid as CAA. Both elevated Aβ levels and CAA can enhance pro-ischemic processes such as vasoconstriction, platelet activation, thrombin formation, fibrin persistence and cerebral vascular cell dysfunction that can culminate in reduced

cerebral blood flow and vessel occlusion. Alternatively, increased Aβ and CAA can promote cerebral vascular cell death and the activation of numerous proteolytic pathways involving MMPs and plasminogen activators that can lead to loss of vessel wall integrity and hemorrhage. However, it should be noted that each of these processes and pathways are not mutually exclusive for either ischemia or hemorrhage and that crosstalk between these cerebral vascular injuries likely occurs. Further, the delicate balance of cerebral hemostasis may be tilted one way or the other based on the location, timing and severity of AβPP, Aβ and CAA.

In any case, there is compelling experimental evidence that Aβ and its precursor AβPP can indeed influence cerebral hemostasis. Nevertheless, due to the complexity of the hemostatic balance further work is needed to better understand how meaningful each of these potential influences are with regards to normal cerebral vascular physiological function and, importantly, pathological cerebral vascular events including ischemic and hemorrhagic stroke.

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HIGHLIGHTS

- Ischemic and hemorrhagic stroke contribute to cognitive impairment and dementia
- Ischemic and hemorrhagic stroke can occur due to altered cerebral hemostasis
- Amyloid β-protein and its precursor can accumulate in cerebral blood vessels
- Amyloid β-protein can modulate cerebral thrombosis and fibrinolysis
- Amyloid β-protein precursor can inhibit cerebral thrombosis

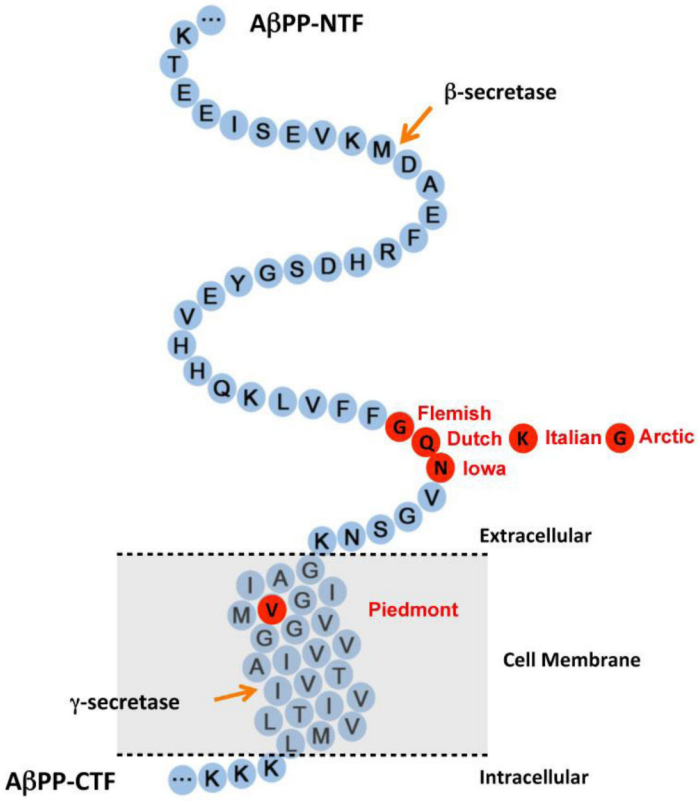

Fig. 1. Location of familial CAA mutations in AβPP

The familial CAA mutations in AβPP (shown in red) all reside within the Aβ domain and tend to cluster in the mid-region of the peptide between residues 21–23 with the exception of the Piedmont mutation that resides on residue 34 of Aβ.

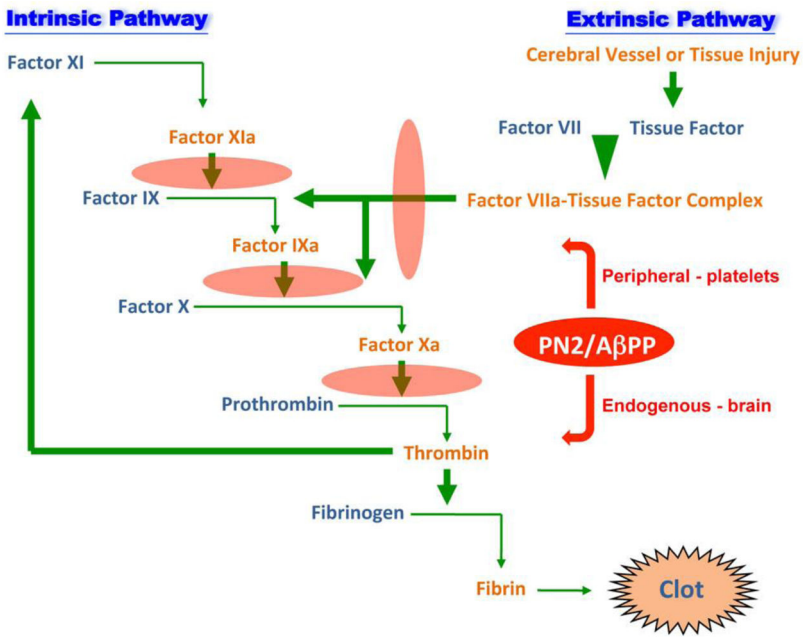

Fig. 2. Physiologic coagulation cascade leading to the generation of thrombin and fibrin clot formation in the CNS: Influence of PN2/AβPP

The pro-thrombotic pathways leading to fibrin clot formation in the CNS are summarized and the steps inhibited by PN2/AβPP are identified. Upon damage to cerebral vessels or tissue the extrinsic pathway is activated with presentation of abundant CNS tissue factor, which binds factor VII to form a proteolytically active Factor VIIa:tissue factor complex. This can lead to the sequential activations of factor IX and factor X leading to the conversion of pro-thrombin to thrombin and ultimately cleavage of fibrinogen to fibrin to form the vascular clot. There is additional amplification of this process through the intrinsic pathway whereby thrombin can activate factor XI. PN2/AβPP, provided either endogenously from the brain or peripherally via circulating blood platelets, can intervene at key steps along these pathways reducing the extent of clot formation.

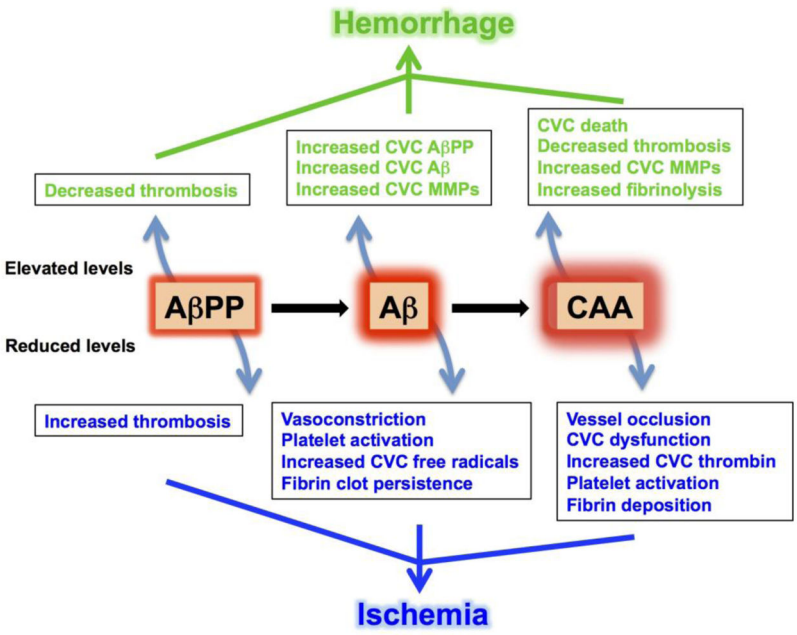

Fig. 3. Influence of AβPP, Aβ and CAA on cerebral vascular pathology and cerebral hemostasis

Increased expression of AβPP and production of Aβ results in formation of CAA. Each of these components can generate consequences that promote and ischemic or hemorrhagic environment in cerebral blood vessels. Increased AβPP, Aβ and CAA can reduce thrombosis, increase cerebral vascular cell (CVC) AβPP and Aβ production, cause CVC death and enhance expression of MMP and plasminogen activator proteolytic systems leading to loss of vessel wall integrity and hemorrhage. Alternatively, elevated Aβ levels and CAA cause vasoconstriction, thrombin production, platelet activation, fibrin deposition and cerebral vessel dysfunction contributing to an ischemic environment in the brain.

Table 1

Inhibition constants for coagulation proteinases and PN2/AβPP.

Proteinase	K_i **[M]**
Thrombin	not inhibited
Factor IXa	$1.9 \pm 0.5 \times 10^{-9}$
Factor Xa	$1.9 \pm 0.7 \times 10^{-8}$
Factor VIIa:Tissue Factor	$7.8 \pm 0.3 \times 10^{-8}$
Factor XIa	$2.9 \pm 0.4 \times 10^{-10}$
Factor XIa + *Zinc*	$6.9 \pm 0.4 \times 10^{-11}$
Factor XIa + *Heparin*	$5.5 \pm 0.3 \times 10^{-11}$
Factor XIa + *Fibrillar Aβ*	$2.8 \pm 0.2 \times 10^{-11}$