Cerebral amyloid angiopathy and the fibrinolytic system: is plasmin a therapeutic target?

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Tables 2; **Figures** 1

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Abstract

Cerebral amyloid angiopathy is a devastating cause of intracerebral haemorrhage for which there is no specific secondary stroke prevention treatment. Here we review the current literature regarding CAA pathophysiology and treatment, as well as what is known of the fibrinolytic pathway and its interaction with amyloid. We postulate that tranexamic acid is a potential secondary stroke prevention treatment agent in sporadic CAA, although further research is required.

Abbreviations:

cerebral amyloid angiopathy (CAA)

hereditary cerebral amyloid angiopathy (HCAA)

amyloid-beta (Aβ)

intracerebral haemorrhage (ICH),

apolipoprotein E (ApoE)

CAA-related inflammation (CAARI)

magnetic resonance imaging (MRI)

computed tomography (CT)

amyloid precursor protein (APP)

Alzheimer's disease (AD)

blood brain barrier (BBB)

matrix metalloproteinases (MMP)

tissue plasminogen activator (tPA)

recombinant tissue plasminogen activator (rtPA)

urokinase plasminogen activator (uPA)

tranexamic acid (TXA)

cerebrospinal fluid (CSF)

Introduction

Cerebral amyloid angiopathy (CAA) is caused by pathologic deposition of amyloid-beta (Aβ) within the vessels of cortical and leptomeningeal arteries, arterioles and capillaries[1]. CAA results in intracranial bleeding in the form of intracerebral haemorrhage (ICH), microbleeds, convexity subarachnoid haemorrhage and superficial siderosis[2]. Most cases of CAA are sporadic as opposed to hereditary, but there are some well-documented examples of genetic forms of CAA in families (so called Hereditary or HCAA)[3]. The prevalence of CAA increases with age, and it is an important cause of both stroke due to ICH and dementia. Despite increasing research, there are currently no therapeutic agents that target the pathophysiology of CAA in order to prevent potentially devastating haemorrhagic complications including ICH.

Proposed mechanisms for increased deposition of Aβ in CAA primarily relate to impaired clearance of amyloid via perivascular drainage pathways and enzymatic breakdown[4]. Increased amyloid deposition within the superficial cerebral small vessels results in haemorrhage via a number of proposed mechanisms including physical disruption of microvascular architecture, activation of matrix metalloproteinases (MMP) and disruption of the blood brain barrier (BBB). In this paper, we describe a novel potential mechanism involving the fibrinolytic system in the pathogenesis of CAA-related ICH. Finally, we will discuss targeting the fibrinolytic system through the use of tranexamic acid (TXA), a commonly used antifibrinolytic, as a possible secondary stroke prevention treatment in CAA.

Sporadic cerebral amyloid angiopathy

Epidemiology and risk factors

The incidence of CAA is strongly age-dependent, with increased Aβ deposition seen from the sixth decade[5]. CAA prevalence increases with age and disease is highest in people with dementia; population-based autopsy studies indicate a CAA prevalence in 80 to 90 year old patients of 20 to 40% in non-demented, and 50 to 60% in demented patients[6].

Apolipoprotein E (ApoE) alleles, specifically ApoE ε4, are the only known genetic risk factors for sporadic CAA. ApoE is a protein which regulates lipid metabolism, and although the mechanism by which it

affects risk of CAA is not well understood, it is postulated that it modulates amyloid metabolism and accumulation[7].

Clinical presentation

Patients with CAA most commonly present with symptomatic ICH, with CAA being a common cause of lobar ICH in older adults, and the second most common cause of ICH after deep perforator (hypertensive) arteriopathy[8]. The distribution of ICH in CAA relates to the distribution of Aβ-containing vessels, with a cortical-subcortical distribution that generally spares the deep white matter, basal ganglia and the brainstem[9]. Other than symptomatic ICH, mild cognitive impairment and dementia, transient focal neurological symptoms and convexity subarachnoid haemorrhage are also recognised presentations of CAA[10]. As such, CAA can be considered as a spectrum disorder with a range of clinical presentations. Patients with CAA-related inflammation (CAARI), an uncommon subset of the disease, may present with seizures, headaches, focal neurological signs and subacute encephalopathy[3].

Diagnosis

The clinical diagnosis of CAA is based on the modified Boston criteria. As shown in table 1, these criteria define definitive, probable and possible CAA based on clinical symptoms, radiographic findings and pathologic specimen if available[2]. Definitive diagnosis is made by histologic examination, but this is rarely undertaken in clinical practice outside of post-mortem or surgical ICH evacuation. The best validated magnetic resonance imaging (MRI) correlates of CAA are cerebral microbleeds in a strictly lobar distribution[11] and cortical superficial siderosis[2]. Other associated MRI features are discrete subcortical white matter hyperintensities[12], deep leukoencephalopathy, expanded perivascular spaces in the white matter[13], convexity subarachnoid haemorrhage and asymptomatic or silent, acute tiny infarcts[14].

Computed tomography (CT) based criteria were developed given MRI imaging is not always widely available and that some CAA patients may be unable to undergo MRI scanning. The Edinburgh criteria, shown in Table 2, use CT findings and ApoE ε4 positivity to rule out CAA-associated lobar ICH[15]. These criteria have only been validated for severe (fatal) ICH and have not been compared to the Boston criteria.

Diagnostic criteria are currently being revised which may improve their sensitivity[16] with important implications for future clinical trials in CAA

Pathophysiology

Deposition of Aβ in CAA occurs characteristically in the media and adventitia of small and medium-sized vessels, arterioles and capillaries of the cerebral cortex, subcortex and leptomeninges[17]. In contrast to Alzheimer's disease (AD), where Aβ42 is deposited in the brain parenchyma, Aβ40 is the more common soluble form of amyloid found in vessel walls causing CAA. Deposition is typically lobar, seen especially in the occipital lobe, as well as the parietal, frontal and temporal lobes, while deep structures such as the basal ganglia and hippocampus are often spared[17].

The source of cerebrovascular amyloid has not been clearly elucidated. There are two main and arguably complementary hypotheses. The first is that Aβ is generated by neurons. This is supported by several mouse models which generate Aβ under a neuronal promoter [18]. In one model of HCAA, mice have neuronal overexpression of E693Q amyloid precursor protein (APP)[19]. These mice show similar pathology to sporadic CAA with amyloid deposition in the cerebral vasculature, rather than the brain parenchyma. Given these mice only have neuronal overexpression of the mutated protein, it was suggested that neurons were the source of cerebrovascular amyloid in this model.

A second hypothesis, although controversial, is that systemic amyloid crosses the BBB and deposits in cerebral vessels. This hypothesis is supported by a 2010 study in which a mouse model of AD received intraperitoneal inoculation of Aβ-rich material, resulting in cerebral amyloidosis[20]. It is important to recognise here that this mouse model was of AD and focused on cerebral amyloidosis rather than cerebrovascular amyloid deposition.

CAA is a progressive disease, and Aβ deposition in the cerebral vasculature is thought to be caused by impaired clearance mechanisms rather than overproduction leading to the concept of a "protein elimination failure angiopathy"[21]. Firstly, the setting down of Aβ in the cerebral vessels maps the intramural

perivascular drainage pathways[22]. This perivascular transport system allows interstitial fluid and solutes to drain in and out of the brain via the basement membranes of capillaries[23] and between smooth muscle cells in the tunica media of small arteries; a process that is proposed to be driven by vessel pulsations[24]. Impairment of these pathways may reduce Aβ clearance in CAA as the character of vessel pulsations changes with Aβ deposition and age (as arteriosclerosis) increases vessel stiffness. Secondly, the composition of the basement membrane and therefore its function has also been shown to change with possession of ApoE ε4 and increasing Aβ deposition, further impairing Aβ clearance[25]. Finally, cerebral Aβ undergoes enzymatic degradation via numerous proteolytic pathways and clearance then occurs via the low-density lipoprotein receptor-related protein-1 and phagocytosis by perivascular macrophages, astrocytes and microglia. Ageing and possession of ApoE ε4 adversely impact all of these mechanisms[10].

Proposed mechanisms of ICH in CAA

Physical disruption of microvascular architecture is the most commonly proposed mechanism of ICH in CAA due to increased amyloid deposition in cerebral vessels. In early disease, Aβ is deposited in the outermost portion of the tunica media surrounding smooth muscle cells and the adventitia. With increased cerebrovascular amyloid deposition, and thus severity, acellular thickening of the vessel wall occurs with all layers of the vessel wall showing Aβ deposition with loss of smooth muscle cells[4]. In severe disease, there is disruption of the vascular architecture which may result in fibrinoid necrosis, focal vessel wall fragmentation and microaneurysm formation[26]. This results in vessel fragility and rupture, predisposing the patient to blood vessel leakage (microhaemorrhages) or frank haemorrhage. There is data that challenges this theory however, with post-mortem histopathological analysis of 9 cases showing reduced Aβ density surrounding areas of microhaemorrhage compared to control areas[27].

Disruption of the BBB and active inflammation as a result of cerebrovascular Aβ have been hypothesised to potentially contribute to pathogenesis[4]; however this has not been well explored experimentally. Post-mortem studies of human CAA brain tissue have suggested evidence of BBB leakage with increased extravasation of fibrin and IgG into the cortex[28], and increased fibrinogen in patients with CAA and AD compared to controls[29].

Activation of MMPs, specifically, MMP-2 and MMP-9 has also been implicated in human and transgenic mouse models as a cause for spontaneous ICH in CAA[30, 31]. MMP-2 and MMP-9 expression, release and activation are induced by Aβ resulting in degradation of the extracellular membrane, disruption to the BBB and therefore increased risk of ICH.

Impaired dynamic cerebral autoregulation was found in a recent CAA study in which the degree of impairment was associated with the number of cerebral microbleeds[32]. Cerebral autoregulation provides a protective buffer mechanism against the changes in systemic blood pressure.

Current treatment options in CAA

There are no proven management options that stop or reverse Aβ deposition in CAA. Current management focuses on secondary prevention, with risk factor modification including treatment of hypertension through lifestyle modifications (smoking cessation, improved diet and exercise) and the use of anti-hypertensives[26], with studies showing an increased risk of haemorrhage with uncontrolled hypertension[33]. The PROGRESS trial CAA sub-study showed blood pressure lowering reduced the risk of stroke (ischaemic and haemorrhagic) by 77%[34]. Avoidance of anticoagulant and antiplatelets agents is generally advised as their use has been shown to increase ICH risk[35], although this was not confirmed in the recent RESTART trial where antiplatelet agents where evaluated in patients with occlusive vascular disease[36]. To date, there have been no randomised controlled trials of secondary stroke prevention specifically in CAA.

Trials to prevent primary ICH in CAA have sought to target amyloid accumulation. Ponezumab is a monoclonal antibody that targets an epitope encompassing the C-terminal amino acids of the Aβ1–40 peptide derived from the human APP. In one study, it has been shown to reduce amyloid burden in transgenic mice with prominent CAA. The primary endpoint of the study, a change in cerebrovascular reactivity as assessed on functional MRI, was not met and efficacy was not shown. The total number of new cerebral microbleeds from baseline did not differ between groups and there were no comments on the clinical effect as no assessments of cognitive change were made[37]. Further trials with ponezumab are not being pursued.

A phase 2 trial investigating the use of tramiprosate, the amino acid homotaurine, has also been undertaken. The rationale was that in vitro studies have shown inhibition of Aβ formation[38] by tramiprosate. No safety concerns were raised; but due to lack of evidence of efficacy the drug has not been developed further for CAA.

The fibrinolytic system and neurological disease

The fibrinolytic system involves the conversion of the abundant plasma protein plasminogen to its potent active form plasmin, via tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA) as shown in Figure 1. Both tPA and plasminogen contain important lysine binding sites located in key structural domains (known as "kringles") that bind to exposed lysine residues on the fibrin surface. This binding allows for the co-localisation of both tPA and plasminogen to the intended substrate (i.e. fibrin). This in turn allows for plasmin to be generated precisely where it is needed and with minimal off-target degradation of other plasma proteins. This lysine-dependent interaction is therefore a fundamental event underpinning fibrinolysis.

The role of fibrin

Fibrin deposition is seen in autoimmune and neurodegenerative diseases such as multiple sclerosis[39] and AD and is associated with increased BBB disruption, innate immune system activation[40] and oxidative injury resulting in neurodegeneration[41]. A monoclonal antibody that targets a fibrin epitope has been explored in mouse models of these diseases and was shown to inhibit autoimmunity- and amyloid-driven neurotoxicity[39].

The amyloid peptide has also been shown to bind to fibrin and inhibit fibrinolysis in vitro[42]. Fibrin co-localises amyloid in the AD brain making clots more resistant to thrombolysis[42], while depletion of fibrin accelerates neuroinflammation and cognitive decline in animal models of AD[43]. Paradoxically, plasminogen deficiency, and the use of TXA, in mouse models of multiple sclerosis has also been shown to delay disease onset[44], possibly by modifying the neuroinflammatory demyelination. This protective mechanism seemingly outweighed the anticipated deleterious effect due to the increase in fibrin accumulation.

Fibrinolytic system and amyloid

There are a number of increasingly recognised actions of the fibrinolytic system that have little to do with fibrin removal. Misfolded and necrotic proteins produced from neuronal injury, including Aβ[45], promote plasmin generation also via interaction of plasminogen with exposed lysine residues presented on these proteins; identical to the process that occurs when plasminogen binds to fibrin [46]. The fibrinolytic system is a key process that removes misfolded proteins, with fibrin (that contains amyloid features) being an important, but not sole target for plasmin proteolysis.

Plasmin can also be proinflammatory by promoting chemotaxis[47], cytokine release, and activation of cell signalling[48]. These actions occur via the ability of plasminogen to bind to its receptors on immune cells. There are 12 identified plasminogen receptors[49], most of which interact with plasminogen via C-terminal lysine receptors. Hence lysine analogues (such as TXA), can also block many cell surface interactions and downstream intracellular signalling events initiated by the plasminogen activating system.

Another important role of the fibrinolytic system is in relation to its effect at modulating BBB permeability which can lead to spontaneous ICH. The best example of this is seen in patients with ischaemic stroke following rtPA thrombolysis where ICH can occur in ~6.5% of cases[50]. It is now known that this capacity of rtPA to promote ICH involves several of its actions on the BBB via plasmin-dependent and independent mechanisms[51].

Relationship of the fibrinolytic system to CAA-induced ICH

In CAA, fibrin has been shown to be associated with BBB leakage, with one study suggesting fibrin-positive vessels, but not amyloid positive vessels, were a better marker of BBB disruption as indicated by cerebral microbleeds on MRI[28]. The interaction of fibrin with mutated Aβ in HCAA has been shown to enhance tPA-mediated plasminogen activation, alter clot structure and delay fibrinolysis; and paradoxically was also suggested to contribute to haemorrhagic and ischaemic manifestations of CAA[29]. The pathophysiology of hereditary CAA may differ from that of sporadic CAA, but might also be amenable to targeting of the fibrinolytic system.

The role of the fibrinolytic system in precipitating symptomatic ICH in CAA has not previously been explored therapeutically. As described above, Aβ is a known co-factor through which tPA can activate plasminogen in a lysine dependent manner, similar to fibrin and misfolded proteins[52]. While plasminogen activation would be initiated to help clear both Aβ and fibrin, it is also likely that excessive plasmin generation would also occur due to very high levels of Aβ and this may have other consequences. Upregulation of plasmin in combination with vessel wall damage from Aβ deposition and alterations in brain microvasculature may result in a relatively hyperfibrinolytic environment with an increased risk of spontaneous ICH[52]. This hypothesis is further supported in clinical practice, by increased rates of remote ICH (suggesting a diffuse vasculopathy - associated with the presence and burden of cerebral microbleeds – particularly in a strictly lobar distribution) complicating rtPA thrombolysis (with increased plasmin generation) in acute ischemic stroke[53].

Targeting the fibrinolytic system with tranexamic acid: a possible therapy for CAA

TXA is a synthetic lysine-analogue and a potent anti-fibrinolytic agent that effectively inhibits the activation of plasminogen to plasmin by binding to the lysine binding sites within the kringle domains on plasminogen and tPA. This in turn denies the capacity of plasminogen and tPA to bind to the exposed lysine residues in fibrin and to other misfolded proteins (Figure 1) hence sparing them from plasmin-mediated degradation.

Hence, we propose that blockade of plasmin generation by TXA in CAA patients, will reduce symptomatic intracerebral bleeding. Given the many effects of the fibrinolytic system however, there may be other effects of TXA which would also be beneficial, such a preventing increased permeability of the BBB and reducing inflammation. TXA has been shown to block the ability of tPA to increase BBB permeability in vitro[54], and the use of TXA following a subarachnoid haemorrhage has been shown to reduce the rate of re-bleeding by 35%[55].

Plasmin generation has also been shown to suppress the immune response. This has prompted recent studies to evaluate the immune modulatory properties of TXA in other disease states, including traumatic

brain injury[56]. TXA has been found to reduce post-operative infection rates in a post hoc analysis in the ATACAS trial of TXA in cardiothoracic surgery; a result that was independent of its haemostatic effects and transfusion requirements[57]. Although speculative, the ability of TXA to control the immunosuppressive effects of plasmin may also have potential to alleviate the inflammatory form of CAA, CAARI.

Current clinical use of TXA

TXA is cheap, readily available, heat stable and generally considered safe. It is used in a range of haemorrhagic conditions, including major surgery, traumatic brain breeding, post-partum haemorrhage, heavy menstrual bleeding and inherited bleeding disorders[58]. The use of TXA has been investigated in cerebrovascular disease in the TICH-2 trial, assessing efficacy in ICH[59]. TXA was administered intravenously, with 1g given initially, then a further 1g given over 8 hours to patients presenting to hospital within 8 hours of symptom onset with spontaneous ICH attributed to small vessel disease. There was no significant difference between the TXA and placebo groups in terms of functional status at day 90, however reductions in secondary endpoints of early death and haematoma expansion were seen. On subgroup analysis, there was a weak signal that TXA was more effective for lobar than deep ICH.

Safety of TXA

There are three important concerns when considering the possible utility for TXA to prevent ICH in CAA – the theoretical risk of thromboembolic complications, seizures and increasing amyloid deposition.

Most large-scale randomised control trials[57, 59-61] and a meta-analysis[62] have shown no increased risk of thromboembolic events in the TXA groups although TXA administration was usually for less than 24 hours. There was a small increase in venous thromboembolic events in the TXA group in the HALT-IT trial, where TXA was evaluated in patients with gastrointestinal bleeding, although arterial thromboembolic events were similar[61]. Specifically related to an older, co-morbid population, the TICH-2 trial showed that TXA caused no increase in venous thromboembolism[59].

Any consideration of TXA as a treatment modality for CAA would require chronic treatment, perhaps for years. The safety profile of such long-term treatment, particularly in relation to thromboembolic risk, is unknown. Its use was shown to be safe in women with heavy menstrual bleeding taking 1.3g of TXA three times daily for 5 days for 27 menstrual cycles. The main adverse effect reported was nausea. No thrombotic or thromboembolic adverse effects were reported[63].

The risk of cerebral thrombotic events is an important consideration because micro-infarcts on MRI are well documented in CAA. The mechanism hypothesised is related to ongoing vasculature injury, with sub-cortical small hyperintensities seen on diffusion weighted MRI[64]. The clinical significance of these ischaemic lesions is unclear. While inhibition of fibrinolysis potentially poses a risk for thrombosis, any risk may further be complicated by recent findings showing that Aβ mutations can promote cerebral fibrin deposits. This was shown to occur by increased binding affinity for fibrinogen[29]. In addition, small studies have reported that plasma Aβ binds to fibrinogen and fibrin, producing clots that are structurally abnormal and harder to degrade[65]. The risk of thrombosis and thrombo-embolism with long term antifibrinolytic treatment such as TXA remains uncertain, especially in the setting of CAA.

Although very rare, persistently high concentrations of TXA are associated with the risk of seizures in the operative (0.7% in the ATACAS trial)[57] and non-operative setting[66]. Most studies have administered TXA intravenously, and high doses of 80 to 100mg/kg have been shown to independently increase the risk of seizures[67]. The mechanism by which this occurs is hypothesised to be related to TXA causing hyperexcitability through reducing inhibition in the CNS[66]. In using TXA as secondary prevention of ICH in CAA, CSF concentrations of TXA would have to be high enough to be therapeutic while balancing the risk of potential seizures, in an already at risk brain.

Based on plasmin's role in facilitating clearance of misfolded proteins, targeting the plasmin-generated mechanisms of ICH in CAA with TXA may theoretically impair amyloid and fibrin clearance and increase extravascular deposition – with possible implications for both cerebrovascular disease and cognitive decline. This is an important consideration. Indeed, increases in plasmin proteolysis in mice (by reducing levels of

plasminogen activator inhibitor-1) has been reported to enhance Aβ clearance[68]. However, other amyloid clearance mechanisms are likely to play an important role, as plasminogen deficient mice have not shown increases in baseline levels of amyloid in either plasma or brain extracts[69]. It should be noted that this finding occurred under non-pathological conditions, and not in mice predisposed to Aβ deposition. In addition, it is unlikely TXA would affect other mechanisms of amyloid degradation, such as the ubiquitin–proteasome system or through autophagy-lysosome. These mechanisms are less involved in BBB breakdown and ongoing amyloid clearance would still occur[70], potentially minimising this unintended consequence, although data is lacking. Nevertheless, cognitive decline would need to be evaluated in any trials investigating the use of TXA in CAA.

Conclusion

Sporadic CAA is a common disease and an important contributor to morbidity and mortality in older people due to clinical manifestations of spontaneous ICH and cognitive decline. Its incidence will continue to increase due to the aging global population and given there are no treatment options currently available to prevent CAA-related ICH, CAA will be an ever-increasing burden on healthcare systems. Improved understanding of CAA pathophysiology is needed to develop treatment targets, with previous studies exploring the use of expensive monoclonal antibodies. Here, we have proposed the fibrinolytic system as a potential mediator and treatment target of CAA-related ICH and set out a rationale for the use of TXA, an inexpensive and seemingly well-tolerated medication that has been shown to cross the BBB. In order to explore the use of TXA further for secondary prevention of the devastating outcome of ICH in sporadic CAA, more research is needed to address potential safety concerns whilst gaining estimates of any clinical effect.

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Legends

Table 1: Modified Boston criteria for diagnosis of CAA, adapted from Linn et al (2**)**

Table 2: The Edinburgh CT and genetic diagnostic criteria for lobar ICH associated with CAA, adapted from Rodrigues et al (15)

Figure 1: Plasminogen binds to fibrin by binding to exposed lysine residues (lys) via 4 lysine binding sites (red dots) located in the kringle (K) domains of plasminogen. The same process occurs when plasminogen binds to misfolded proteins or amyloid beta. tPA generates plasmin and results in protein degradation. Interaction of plasminogen with lysine residues in either fibrin or misfolded proteins/amyloid beta is blocked by TXA. Excessive plasmin generation in CAA due to presence of amyloid deposits may increase the risk of ICH and promote neuroinflammation.

Table 1

	Modified Boston criteria
Definitive CAA	Full post-mortem examination demonstrating: - Lobar, cortical or corticosubcortical haemorrhage - Severe CAA with vasculopathy - Absence of other diagnostic lesion
Probable CAA with supporting pathology	Clinical data and pathologic tissue demonstrating: - Lobar, cortical or corticosubcortical haemorrhage - Some degree of CAA in specimen - Absence of other diagnostic lesion
Probable CAA	Clinical data and MRI or CT demonstrating: - Multiple haemorrhages restricted to lobar cortical or corticosubcortical haemorrhage OR single lobar, cortical or corticosubcortical haemorrhage OR disseminated superficial siderosis - Age ≥55 years - Absence of other cause of haemorrhage or superficial siderosis
Possible CAA	Clinical data and MRI or CT demonstrating: - Single lobar, cortical or corticosubcortical haemorrhage OR focal or disseminated superficial siderosis - Age ≥55 years - Absence of other cause of haemorrhage or superficial siderosis

Table 2

	Edinburgh criteria
High probability CAA	Lobar ICH showing subarachnoid haemorrhage on CT and either: - Finger-like projections from the ICH on CT, or - Possession of at least one ApoE ε4 allele
Intermediate probability CAA	Lobar ICH showing either - Subarachnoid haemorrhage on CT, or - Possession of at least one ApoE ε4 allele
Low probability CAA: rule out criteria	Lobar ICH showing neither subarachnoid haemorrhage on CT nor possession of at least one ApoE ε4 allele

Figure 1: Schematic representation of the fibrinolytic system and how it can potentially promote ICH in CAA