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Amyloid- β may keep clots away

Gregory J del Zoppo

Professor of Medicine in Hematology and Adjunct Professor of Neurology at the University of Washington, Harborview Medical Center, Seattle, Washington, USA. grgdlzop@u.washington.edu

Deposition of amyloid in the parenchymal tissues and the blood vessels of the brain accompanies Alzheimer's disease. In the elderly, vascular deposition of amyloid can also have another impact: it can be associated with brain hemorrhage. Amyloid deposition increases the risk for small hemorrhages, dubbed micro-hemorrhages, and for a particular type of spontaneous hemorrhage within the gray matter of the brain, called a lobar hemorrhage. But not all individuals who suffer from amyloid deposition are at risk for hemorrhage (Fig. 1). One possibility is that the risk depends on the type of amyloid deposited.

Findings within the past few years have hinted that a domain that inhibits proteases, called a Kunitz protease inhibitor (or KPI) domain, found in several isoforms of β -amyloid ($A\beta$), inactivates proteins in the blood that cause clotting^{1–5}.

Does this inactivation underlie the link between amyloid and hemorrhage? Two recent studies by Xu *et al.*^{1,2} bolster this hypothesis. In one study, the authors found that mice constructed to make increased amounts of the precursor protein to $A\beta$ peptide ($A\beta$ PP) had larger hemorrhages than wild-type mice in a model of artificial brain hemorrhage¹. In a second study, mice that did not make $A\beta$ PP experienced smaller hemorrhages in the same model². What's more, the isolated KPI domain could block coagulation^{1,2}. The two studies suggest that, with aging, deposition of some isoforms of $A\beta$ in brain microvessels may increase the chance of hemorrhage by blocking coagulation locally.

Previous observations have suggested that the location of amyloid seems to influence the location of the hemorrhage. In blood vessels, amyloid deposits are found predominantly in the walls of arterioles and arteries in the cortex and deep brain structures. This location explains the frequent lobar distribution of the amyloid-associated hemorrhages. $A\beta$ PP is found less often in capillaries and veins, but when it is, $A\beta$ PP infiltrates the matrix that forms the basal lamina, as well as the surrounding tissue. This deposition interferes with normal vessel function and may also lead to microhemorrhages.

Another condition associated with aging, ischemic stroke, may also play into the risk of amyloid-associated hemorrhage. Hemorrhage occurs in the injury regions in up to 65% of ischemic strokes. Deposits of $A\beta$ peptide are seen in cerebral vessels of some individuals after ischemic stroke. Ischemia alters the adhesion of endothelial cells and astrocytes to the intervening basal lamina^{6,7}, and it can also promote thrombosis within brain microvessels⁸. One possibility is that $A\beta$ peptides change cell-matrix interactions within brain microvessels and cause their instability, thereby promoting hemorrhage when the vessels are injured.

The notion that $A\beta$ peptides themselves might directly promote hemorrhage by inhibiting thrombosis gained traction with studies of KPI domains. $A\beta$ PP, the precursor of $A\beta$, occurs in numerous isoforms, a result of alternative splicing of a single gene. Two isoforms of $A\beta$ PP have KPI domains that do not occur in other forms of the protein. Kunitz domains have a characteristic active site and a typical double-loop structure that is conserved among proteins in which KPI domains are found. One or more such domains are found in biologically active proteinase inhibitors, including tissue factor pathway inhibitor, aprotinin, and the $A\beta$ PP^{9,10}.

Inhibitors with Kunitz-like folds, or KPI-containing inhibitors, are employed by many species to regulate the initiation of clotting that prevents hemorrhage from a vessel (hemostasis) or to block coagulation at specific steps (such as thrombin formation). Ticks also use such domains to block blood coagulation^{9–11}. In addition, peptides derived from the KPI domain can have antihemostatic activity.

One isoform of A β PP found in cerebral blood vessels and in brain deposits in individuals with Alzheimer's disease has a KPI domain identical to protease nexin-2 (PN-2), a protein known to regulate hemostasis¹². *In vitro* studies have suggested that A β PP/PN-2 (the PN-2-identical isoform of A β PP) inhibits the coagulation factors IXa and XIa^{10,11}, which, by blocking thrombosis, could promote hemorrhage. Xu et al.^{1,2} now flesh out this model.

The researchers examined transgenic mouse strains with either increased expression of a KPI-containing isoform of A β PP in platelets or in neurons¹. They subjected the mice to a standardized experimental hemorrhage, made by injecting a fixed amount of the matrix protease collagenase into the brain¹. The volume of hemorrhage and hemoglobin deposition, they found, was substantially higher in the transgenic mice than in wild-type mice^{1,5}. The authors also examined occlusion in the carotid artery in a model of laser-induced arterial thrombosis and found that occlusion occurred more rapidly in the mice expressing the KPI-containing A β PP/PN-2 in platelets compared with wild-type mice or mice over-expressing the KPI-containing A β PP/PN-2 in neurons¹. These findings suggest that the A β PP KPI insert could be responsible for enlargement of the cerebral hemorrhage in the model.

Xu et al.² also examined mice engineered not to express A β PP and its KPI domain. They compared these mice to wild-type mice in the hemorrhage model and found substantially smaller cerebral hemorrhages with less hemoglobin deposition². Similarly, mice lacking the amyloid precursor-like protein-2 (APLP-2), which has a KPI domain, showed a decreased volume of hemorrhage compared to wild-type mice. Experiments in the carotid artery model indicated that when A β PP or APLP-2 were absent thrombosis was enhanced². Again, the KPI domains alone inhibited coagulation *in vitro*.

These findings have implications for aged individuals, but the studies are not without concerns. Models of cerebral hemorrhage are complex. The method used by the investigators degrades collagen in the vascular basal lamina and surrounding tissues to produce a consistent circumscribed hemorrhage. The model is not pathophysiological partly because the collagenase stimulates its own inflammatory cell response and nonvascular cell injury independent of the hemorrhage—limiting the interpretation of experimental results. Models of ischemic stroke that produce hemorrhagic transformation of the injury (where ischemia causes the hemorrhage) would be of interest but are less consistent. Nonetheless, the ability of A β PP/PN-2 to increase the size of the hemorrhage in the model used suggests that some portion of the molecule has an anticoagulant effect, and this seems to be the KPI domain. Intriguingly, mice lacking A β PP/PN-2 had decreased hemorrhage volume in this model, implying either that enhanced thrombosis limited the hemorrhage size or that there are some aspects of the artificial hemorrhage in mice that express A β PP with the KPI domain that are not understood.

Studies building on the new findings could broaden their clinical relevance. But it is important first to know how cerebral microvessels interact molecularly with A β PP and the KPI domain. The matrix barrier in the cerebral microvasculature could well be a partner in these events. Perhaps A β PP can interrupt the interaction of endothelial cells and other components of the microvessel with the basal lamina.

As hemorrhagic transformation is a common accompaniment of focal ischemia, what would be the impact of varying amounts of A β PP with and without the KPI domain on the risk of hemorrhage in the setting of mouse ischemic stroke? Does A β PP/PN-2 contribute to the varying

risk of hemorrhage in individuals after ischemic stroke? Perhaps some people have one A β PP isoform with a KPI domain and others have non-KPI-containing isoforms. Perhaps the various isoforms of amyloid within the cerebral vessels promote different risks of hemorrhage. Maybe the frequency of hemorrhage in individuals with ischemic stroke is linked to the amount of A β deposition.

Knowing whether A β is deposited, and which isoforms are present in people who are at risk for stroke, could help us know who might carry the risk of cerebral hemorrhage.

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Kyra Becker

Figure 1. Amyloid-related cerebral hemorrhages. Magnetic resonance image of the brain from a patient with amyloid deposition disease that displays microhemorrhages in gray matter (arrowheads) and a parenchymal hemorrhage (arrow).