A possible mechanism for the enhanced toxicity of beta-amyloid protofibrils in Alzheimer’s disease


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The amyloid-beta peptide (Aβ) is a driver of Alzheimer’s disease (AD). Aβ monomers can aggregate and form larger soluble (oligomers/protofibrils) and insoluble (fibrils) forms. There is evidence that Aβ protofibrils are the most toxic form, but the reasons are not known. Consistent with a critical role for this form of Aβ in AD, a recently FDA-approved therapeutic antibody targeted against protofibrils, lecanemab, slows the progression of AD in patients. The plasma contact system, which can promote coagulation and inflammation, has been implicated in AD pathogenesis. This system is activated by Aβ which could lead to vascular and inflammatory pathologies associated with AD. We show here that the contact system is preferentially activated by protofibrils of Aβ. Aβ protofibrils bind to coagulation factor XII and high molecular weight kininogen and accelerate the activation of the system. Furthermore, lecanemab blocks Aβ protofibril activation of the contact system. This work provides a possible mechanism for Aβ protofibril toxicity in AD and why lecanemab is therapeutically effective.

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There is strong genetic evidence from autosomal dominant mutations that amyloid-beta peptide (Aβ) can be a causative factor in Alzheimer’s disease (AD). One such mutation that promotes AD is the Arctic mutation which causes increased Aβ protofibril formation (1). Numerous studies have shown that Aβ protofibrils are more neurotoxic than monomers and insoluble fibrils (2). An antibody that binds preferentially to Aβ protofibrils was developed in mice (3) and adapted for human use. Treatment of early-stage AD patients for 18 months with this antibody, lecanemab, clears Aβ plaques from the brain and slows cognitive decline (4). The mechanism of Aβ protofibril toxicity in AD remains unknown.

In addition to Aβ, there is increasing evidence that vascular abnormalities and neuroinflammation contribute to AD pathogenesis. For example, in patients with autosomal dominant AD, vascular pathology appears more than two decades before cognitive decline (5). In sporadic AD, a decrease in cerebral blood flow is the first pathology to emerge (6). However, the mechanisms by which vascular abnormalities and inflammation are initiated in AD and how they might affect neurons are still unclear.

One system that links vascular and inflammatory pathways is the plasma contact system (7). In this system, activation of factor XII (FXII) leads to blood clotting via factor XI (FXI) and inflammation via generation of the proinflammatory peptide bradykinin upon cleavage of high molecular weight kininogen (HK). There is evidence that implicates the contact system in AD pathology (8). For example, AD patient plasma often shows evidence of contact system activation and dementia ratings correlate well with the extent of HK cleavage and bradykinin generation (9–12). Additionally, the plasma contact system can be activated by Aβ and FXII deficiency offers protection in AD mouse models (9, 13).

The forms of Aβ that activate the contact system have not been defined. Therefore, we prepared Aβ monomers, oligomers, protofibrils, and fibrils (Fig. 1A) and tested their effectiveness in activating the contact system. Aβ protofibrils promoted the activation of the contact system as evidenced by HK cleavage and activation of FXII and prekallikrein (PK), whereas the other forms of Aβ did not (Fig. 1B–G). Levels of bradykinin, generated upon HK cleavage, were increased by Aβ protofibrils compared to all other versions of Aβ (Fig. 1H). Aβ protofibrils also induced faster clotting compared to other Aβ forms (Fig. 1A).

We examined the reasons for this specificity by determining whether Aβ protofibrils could bind to components of the contact system. HK bound tightly to Aβ protofibrils but its binding to other Aβ species was weaker (Fig. 1J). We also incubated biotinylated Aβ species with human plasma and precipitated Aβ and its bound proteins with streptavidin. Substantial amounts of FXII and HK coprecipitated with Aβ protofibrils,
but minimal to no FXII or HK was pulled down with other Aβ species (Fig. 1A). These analyses confirmed that Aβ protofibrils bind to HK and FXII.

Since Aβ protofibrils preferentially activate the contact system and are the target of the recent FDA-approved therapeutic antibody lecanemab, we hypothesized that lecanemab might be working in vivo by inhibiting Aβ's activation of this pathway. Lecanemab dramatically inhibited contact system activation induced by Aβ protofibrils as shown by HK cleavage, FXII and PK activation, FXIIa and PKA activities, and bradykinin generation (Fig. 2 A–G). Lecanemab blocked the binding of FXII and HK to Aβ (Fig. 2H), while control human IgG had no effect. Lecanemab also prevented Aβ protofibril-mediated accelerated intrinsic coagulation in normal human plasma (Fig. 2I).

These results bear on two important questions: 1) Why are Aβ protofibrils more toxic than other Aβ species; and 2) Is one of the beneficial effects of lecanemab on AD patients via its inhibition of the plasma contact system?

It is possible that the size of protofibrils is critical for promoting biological effects. In the instance of the contact system, the Aβ protofibril surface might be large enough for both HK and FXII to bind, bringing them in proximity for efficient contact system activation (14). HK circulates in complex with PK or FXI (15, 16). In regard to the HK/PK complex, proximity of HK/PK to FXII could facilitate cleavage of FXII by PK and accelerate the pathway through positive feedback (7).
Fig. 2. Lecanemab prevents Aβ protofibrils from activating the contact system in normal human plasma. Plasma was incubated with or without Aβ42 protofibrils in the presence of vehicle, lecanemab, human IgG control (Hu-IgG), or G2-11, another anti-Aβ antibody. Analyses as in Fig. 1. (A) Contact system activation was examined by Western blotting. (B–D) Quantitation of Western blots. AU, arbitrary units. (E and F) Activity assays for FXIIa and PKa. (G) Bradykinin levels. (H) Analysis of proteins bound to biotinylated Aβ42. Both FXII and HK bound to Aβ42, which was blocked by lecanemab but not by human control IgG. TRF was used as a negative control. Results are representative of three independent experiments in A–G and four independent experiments in H. (I) Intrinsic clotting, n = 8/group. Lecanemab blocked Aβ-induced accelerated clotting. Data were analyzed by one-way ANOVA with Tukey’s multiple comparisons test and are denoted as mean ± SEM. ***P ≤ 0.001, ****P ≤ 0.0001.

Materials and Methods

Blood was collected and plasma was prepared from healthy human donors (n = 8) who provided informed consent. The procedures were approved by The Rockefeller University Institutional Review Board. Aβ1-42 preparation, Aβ-induced plasma contact system activation, pull-down assays, Western blotting, bradykinin ELISA, FXIIa and PKa activity assays, clotting assays, binding studies, and statistical analyses were as previously described (9–13). For details, see SI Appendix.

Data, Materials, and Software Availability. All study data are included in the article and/or SI Appendix.

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