PNAS BRIEF REPORT | NEUROSCIENCE OPEN ACCESS

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

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Edited by Joseph Takahashi, The University of Texas Southwestern Medical Center, Dallas, TX; received June 4, 2023; accepted July 24, 2023

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.

Alzheimer's | beta-amyloid | coagulation

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. 1*A*) 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. 1 *B*–*G*). Levels of bradykinin, generated upon HK cleavage, were increased by Aβ protofibrils compared to all other versions of Aβ (Fig. 1*H*). Aβ protofibrils also induced faster clotting compared to other Aβ forms (Fig. 1*I*).

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. 1*J*). 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,

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Author contributions: Z.-L.C., P.K.S., E.H.N., and S.S. designed research; Z.-L.C., P.K.S., M.C., and E.H.N. performed research; Z.-L.C., P.K.S., E.H.N., and S.S. wrote the paper.

The authors declare no competing interest.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2309389120/-/DCSupplemental.

Published August 28, 2023.

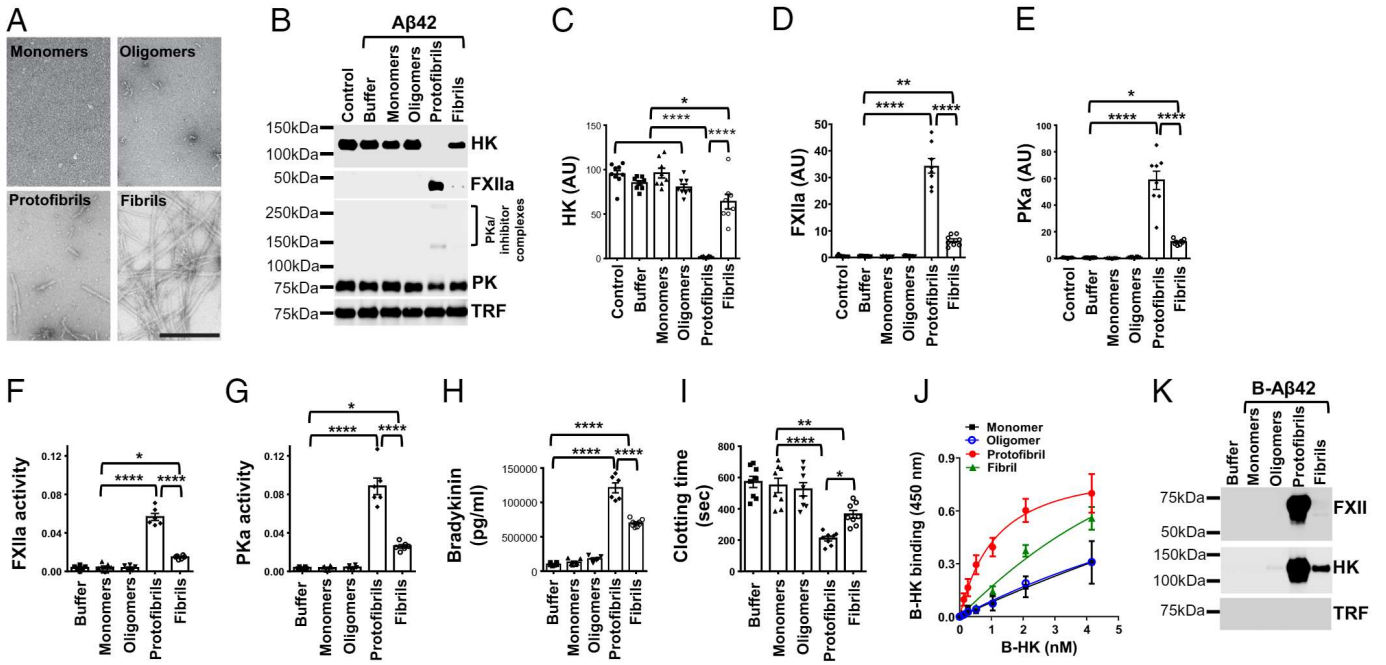

Fig. 1. Aβ protofibrils are the most effective activators of the contact system in human plasma compared to other forms of Aβ. (*A*) Electron micrographs of various Aβ42 species. (Scale bar, 200 nm.) (*B*) Aβ42 was incubated with human plasma, and Western blots were run under reducing conditions. FXII activation was determined using an antibody that detects the activated form, FXIIa. PK activation was determined by the presence of PKa/inhibitor complexes that only form with activated PK. Aβ protofibrils induced complete HK cleavage and activation of FXII and PK. Transferrin (TRF) was a loading control. (*C*–*E*) Quantitation of Western blots. Aβ fibrils induced some contact system activation but significantly less than that of Aβ protofibrils. Aβ monomers and small oligomers did not induce contact system activation. AU, arbitrary units. (*F*) FXIIa and (*G*) PKa activities were measured by chromogenic assays. (*H*) Bradykinin levels were measured by ELISA. (*I*) Clotting time was determined in normal human plasma. Results are representative of three independent experiments. Data were analyzed by one-way ANOVA with Tukey's multiple comparisons analysis and are denoted as mean ± SEM. $*P \le 0.05$, $**P \le 0.01$, $****P \le 0.0001$. (*J*) Binding of biotinylated human HK (B-HK) to Aβ was assessed by ELISA. Binding constants were as follows: Aβ protofibrils/HK, K_D = 1.3 nM; Aβ fibrils/HK, K_D ~ 268 nM. Other binding interactions were too weak to be measured. (*K*) Aβ42 protofibrils pulled down FXII and HK in human plasma, but minimal to no FXII or HK was pulled down with other Aβ species. Transferrin (TRF) was used as a negative control.

but minimal to no FXII or HK was pulled down with other Aβ species (Fig. 1*K*). 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. 2*H*), while control human IgG had no effect. Lecanemab also prevented Aβ protofibril-mediated accelerated intrinsic coagulation in normal human plasma (Fig. 2*I*).

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). A similar logic would apply to the coagulation arm of the contact system: concurrent binding of FXII and HK/FXI could promote more efficient cleavage of FXI by FXII due to close proximity, leading to more coagulation. On the contrary, Aβ monomers and oligomers might be too small to accommodate concurrent binding of both FXII and HK. Fibrils are more elongated and have a larger surface due to lateral association, so the distance between binding partners might be too far and thus limit the interaction between FXII and HK/PK or HK/FXI complexes.

With respect to mechanisms by which lecanemab slows progression of AD, anti-Aβ antibody therapy has been hindered by a serious side effect, amyloid-related imaging abnormalities with edema (ARIA-E) (17). The exact mechanisms for ARIA-E have not been elucidated. One product of contact system activation is bradykinin, a peptide that causes vascular permeability. Overactivation of the contact system can lead to hereditary angioedema, due to excess bradykinin. It is noteworthy that lecanemab causes less ARIA (10%) than other anti-Aβ antibodies such as aducanumab (35%), gantenerumab (30%), or donanemab (27%) (4). Lecanemab's effective blocking of the contact system could reduce bradykinin production and therefore reduce the occurrence of ARIA in patients.

In summary, we present a biological effect of Aβ protofibrils that could help explain their increased toxicity in AD and may also provide insight into the mechanism of lecanemab, a promising anti-Aβ antibody therapy.

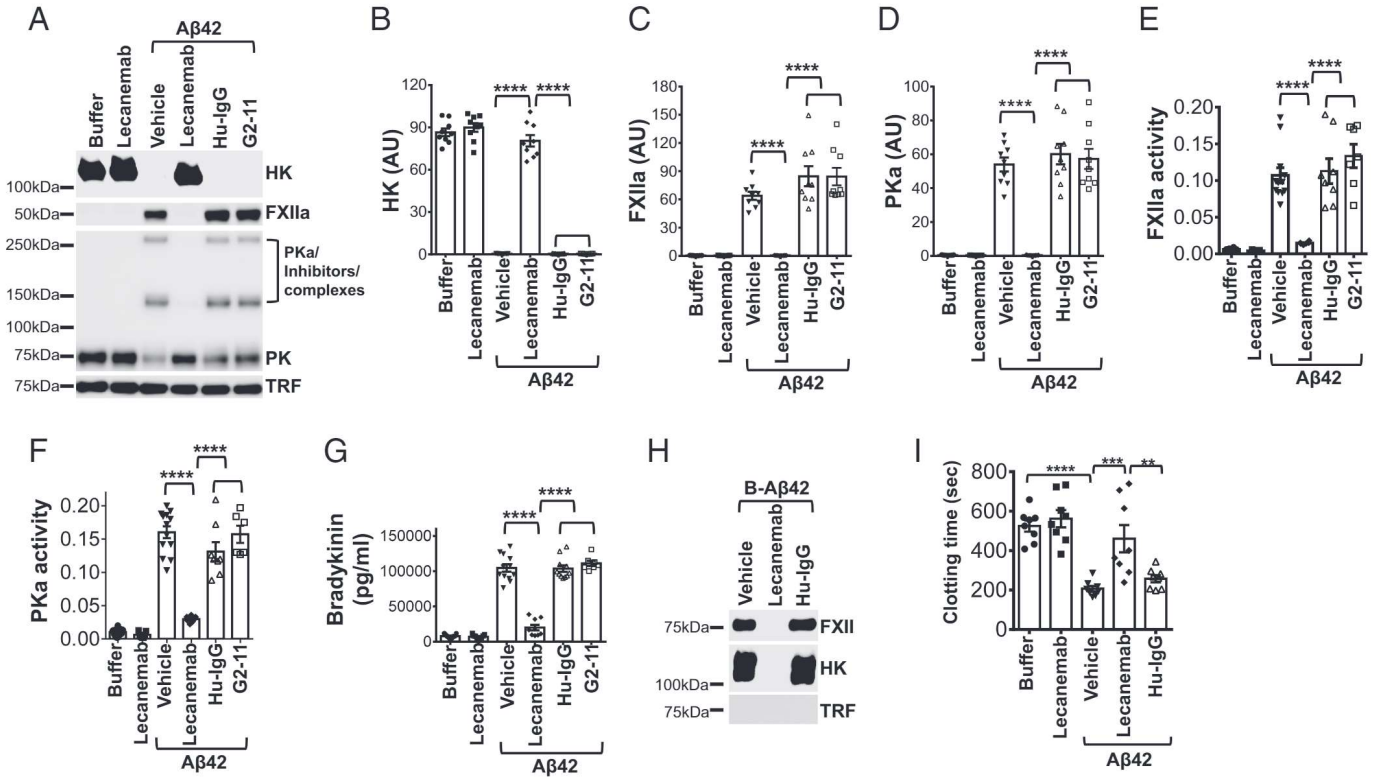

Fig. 2. Lecanemab prevents Aβ protofibrils from activating the contact system in normal human plasma. Plasma was incubated with or without Aβ42 protofibrils in the presence of vehicle, lecanemab, human IgG control (Hu-IgG), or G2-11, another anti-Aβ antibody. Analyses as in Fig. 1. (*A*) Contact system 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. $^{**}P \le 0.01$, $^{***}P \le 0.001$, $^{****}P \le 0.0001$.

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. Aβ preparation, Aβ-induced plasma contact system activation, pulldown 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*.

ACKNOWLEDGMENTS. We thank the members of the Strickland laboratory for helpful discussions. This work was supported by NIH grants NS102721 and AG069987, Robertson Therapeutic Development Fund, Samuel Newhouse Foundation, John A. Herrmann, and May and Samuel Rudin Family Foundation.

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