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Lecanemab blocks the effects of the Aβ/fibrinogen complex on blood clots and synapse toxicity in organotypic culture

Pradeep Kumar Singh[a], Elisa Nicoloso Simões-Pires[a], Zu-Lin Chen[a], Daniel Torrente[a], Marissa Calvano[a], Anurag Sharma[b], Sidney Strickland[a,1], and Erin H. Norris[a,1]

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Proteinaceous brain inclusions, neuroinflammation, and vascular dysfunction are common pathologies in Alzheimer's disease (AD). Vascular deficits include a compromised blood–brain barrier, which can lead to extravasation of blood proteins like fibrinogen into the brain. Fibrinogen's interaction with the amyloid-beta (Aβ) peptide is known to worsen thrombotic and cerebrovascular pathways in AD. Lecanemab, an FDA-approved antibody therapy for AD, clears Aβ plaque from the brain and slows cognitive decline. Here, we show that lecanemab blocks fibrinogen's binding to Aβ protofibrils, preventing Aβ/fibrinogen-mediated delayed fibrinolysis and clot abnormalities in vitro and in human plasma. Additionally, we show that lecanemab dissociates the Aβ/fibrinogen complex and prevents fibrinogen from exacerbating Aβ-induced synaptotoxicity in mouse organotypic hippocampal cultures. These findings reveal a possible protective mechanism by which lecanemab may slow disease progression in AD.

Alzheimer's disease | fibrinogen | lecanemab | amyloid-beta | fibrinolysis

Alzheimer's disease (AD) is a neurodegenerative dementia characterized by the accumulation of amyloid-beta (Aβ) aggregates in the brain parenchyma and in/around blood vessels (cerebral amyloid angiopathy, CAA) (1, 2). An early feature in AD is the disruption of the blood–brain barrier (BBB), which leads to the extravasation and accumulation of blood proteins within the brain, worsening AD pathology (1, 3, 4).

Fibrinogen, an abundant blood protein and major component of blood clots, can form a complex with Aβ upon binding to Aβ's N-terminus (residues 8 to 21) (1, 5). Aβ/fibrinogen complexes may contribute to vascular abnormalities in AD by altering fibrin clot degradation (1). Reducing fibrinogen levels or inhibiting Aβ/fibrinogen binding in AD mice leads to decreased BBB permeability, reduced neuroinflammation, decreased CAA, and less cognitive decline (6, 7).

Lecanemab, an antibody directed against Aβ protofibrils, is an FDA-approved immunotherapy for AD that reduces Aβ burden and slows cognitive decline (8). However, little is known regarding its mechanisms-of-action. Lecanemab targets Aβ's N-terminus (residues 1 to 16), overlapping with fibrinogen's binding site (2, 5). Therefore, we investigated the interaction between Aβ42 and fibrinogen in the absence and presence of lecanemab (Fig. 1 *A*–*C*). Lecanemab blocked Aβ42 binding to fibrinogen in a dose-dependent manner, while human IgG had no effect. The Aβ42 prepared for this study was comprised of curvy linear aggregates (small protofibrils) 30 to 90 nm in length (Fig. 1*D*).

The interaction between Aβ42 and fibrinogen leads to abnormal clot structure that is resistant to plasmin-mediated fibrinolysis (1). Since lecanemab blocked this interaction, we analyzed the effect of lecanemab on Aβ42/fibrinogen-mediated impaired fibrinolysis in a purified protein system. While Aβ42 protofibrils delayed fibrinolysis, lecanemab blocked this effect (Fig. 1 *E* and *F*).

Aβ42 also alters fibrin clot turbidity, an indicator of altered fibrin assembly (7). Lecanemab, but not human IgG, rescued the defect in fibrin assembly caused by Aβ42 protofibrils (Fig. 1 *E* and *G*). We also analyzed clot morphology. As previously reported, Aβ42 disrupted normal clot morphology, causing thinning of the fibrin bundles, abnormal clustering, and entangled clumps (Fig. 1 *H*–*J*). However, in the presence of Aβ42 protofibrils and lecanemab, these structural clot abnormalities were corrected (Fig. 1 *H*–*J*). Human IgG had no effect on Aβ42-induced clot abnormalities (Fig. 1 *H*–*J*).

To determine whether lecanemab inhibits Aβ42/fibrinogen binding ex *vivo*, we incubated biotinylated Aβ42 protofibrils (B-Aβ42) with buffer, lecanemab, or human IgG and added them to normal human plasma (NHP). Immunoprecipitation was performed to pull down any Aβ42-bound proteins. Fibrinogen immunoprecipitated with Aβ42; in the presence of lecanemab, however, Aβ42 did not pull-down fibrinogen, indicating

Author affiliations: [a]Patricia and John Rosenwald Laboratory of Neurobiology and Genetics, The Rockefeller University, New York, NY 10065; and [b]Electron Microscopy Resource Center, The Rockefeller University, New York, NY 10065

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[1]To whom correspondence may be addressed. Email: strickland@rockefeller.edu or enorris@rockefeller.edu.

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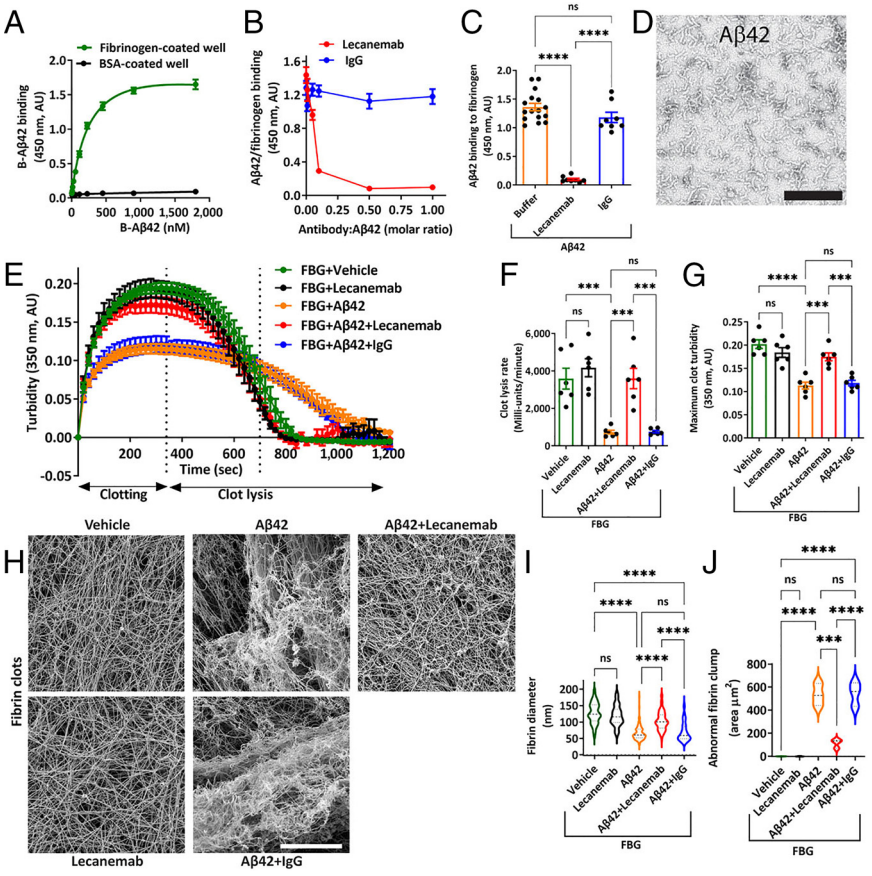

Fig. 1. Lecanemab restores Aβ42-induced delayed fibrinolysis and clot abnormalities by inhibiting the Aβ42/fibrinogen interaction in vitro. (*A*) Biotinylated Aβ42 protofibrils (B-Aβ42) bind to fibrinogen-coated wells. (*B*) Lecanemab dose-dependently blocked the binding of B-Aβ42 to fibrinogen, while human IgG had no effect. (*C*) Quantification of *B* (at equimolar ratio). (*A*–*C*) Data from three independent experiments, n = 8 to 16/group. (*D*) Transmission EM of Aβ42 protofibrils. (Scale bar, 200 nm.) (*E*) Turbidity assay shows clotting and clot lysis phases. Lecanemab, but not control IgG, corrected the Aβ42-induced delayed fibrinolysis. (*F*) Quantification of clot lysis rate in *E* (slope between vertical dotted lines). (*G*) Quantification of maximum clot turbidity in *E*. (*E*–*G*) Data from six independent experiments, n = 6/group. (*H*) Representative scanning EM of fibrin clots from purified fibrinogen with different treatments. (Scale bar, 10 μm.) (*I* and *J*) Quantification of fibrin diameter and total area of abnormal fibrin clumps in clot images. Data from three independent experiments. Vehicle constitutes PBS+DMSO. Comparisons among multiple groups were performed using one-way ANOVA followed by Newman–Keuls multiple comparison test. Data are presented as mean ± SEM. *****P* < 0.0001, ****P* < 0.001; ns, not significant.

that lecanemab blocked Aβ42/fibrinogen binding in NHP (Fig. 2 *A* and *B*). Consistent with in vitro results (Fig. 1 *E*–*J*), lecanemab corrected Aβ42-induced clot abnormalities in human plasma (Fig. 2 *C*–*H*).

Synapse loss in AD is associated with memory impairment (4, 9). For example, the reduction of presynaptic protein synaptophysin (SYP) and postsynaptic density protein-95 (PSD-95) in the hippocampus corresponds to cognitive deficits in AD (10, 11). Extravasated fibrinogen can contribute to synaptic dysfunction (1, 3, 4, 12). Therefore, we explored whether lecanemab could alter fibrinogen's effect on Aβ42-mediated synaptotoxicity by examining the levels of SYP and PSD-95 in mouse organotypic hippocampal cultures (OHC). Treatment of OHCs with a mixture of Aβ42 protofibrils and fibrinogen reduced SYP and PSD-95, while lecanemab inhibited these Aβ42/fibrinogen-mediated synaptic changes (Fig. 2 *I*–*K*).

Moreover, lecanemab dissociated preformed Aβ42/fibrinogen complexes in human plasma (Fig. 2 *L* and *M*) and mitigated synaptotoxicity induced by preformed complexes in mouse OHCs (Fig. 2 *N*–*P*). We have shown that anti-Aβ antibodies whose epitopes overlap with fibrinogen's binding site on Aβ can prevent Aβ/fibrinogen complex formation and dissociate Aβ/fibrinogen complexes (5), so this mechanism is not specific to lecanemab. However, aducanumab, another FDA-approved Aβ immunotherapy (2), did not dissociate Aβ/fibrinogen complexes in human plasma (Fig. 2 *L* and *M*). Therefore, lecanemab's ability to block Aβ/fibrinogen complex formation or dissociate the complex, in addition to its ability to inhibit contact system dysfunction (13), could be some of its protective mechanisms.

The concentration of lecanemab used in our studies is higher than in the CSF of treated AD patients (14), which could be considered a study limitation. However, Aβ42/fibrinogen complexes lead to pathologies both in cerebral blood vessels and the brain parenchyma (1). Therefore, the proposed protective effect of lecanemab against Aβ42/fibrinogen-mediated vascular dysfunction is more likely dependent on the concentration of lecanemab in the patients' blood (8).

Our findings suggest that further investigation into lecanemab's mechanisms-of-action is necessary in AD mouse models and AD patients. Questions remain about lecanemab's efficacy in dissociating and method of clearing Aβ/fibrinogen complexes in vivo and its mechanism in mitigating Aβ/fibrinogen-induced synaptotoxicity. Moreover, given the neurodegenerative impact of extravasated fibrin(ogen) into the brain parenchyma independent of Aβ (1, 3, 4), exploring a combinatorial therapeutic strategy using lecanemab alongside a fibrin-specific antibody (12) or another relevant target could be an effective treatment to improve upon the current AD immunotherapies.

Materials and Methods

Details of reagents and methods are included in *SI Appendix*.

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

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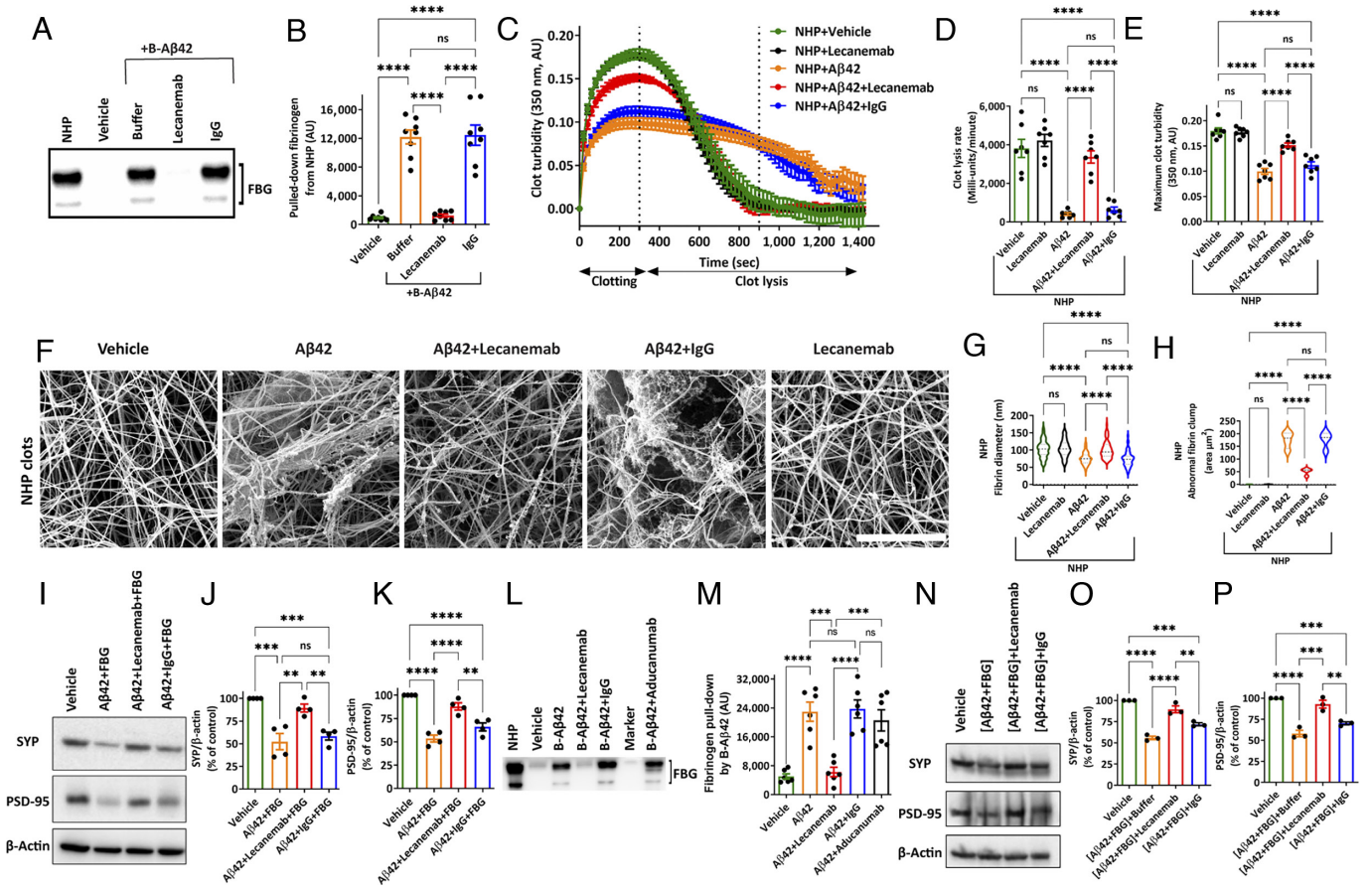

Fig. 2. Lecanemab blocks Aβ42-induced clot abnormalities and delays fibrinolysis in NHP and Aβ42/fibrinogen-mediated synaptotoxicity in mouse OHC. (*A*) Biotinylated Aβ protofibrils (B-Aβ42) immunoprecipitated fibrinogen from NHP but did not in the presence of lecanemab. (*B*) Quantification of *A*. Data from three independent experiments; n = 8/group. (*C*) Clotting and fibrinolysis in NHP using turbidity assay. Lecanemab restored Aβ42-induced delayed fibrinolysis in NHP. (*D*) Quantification of clot lysis rate in *C*. (*E*) Quantification of maximum clot turbidity in *C*. (*C*–*E*) Data from seven experiments, n = 7/group. (*F*) Representative scanning EM of clots formed from NHP with different treatments. (Scale bar, 5 μm.) (*G* and *H*) Analyses of scanning EM showing quantifications of fibrin diameter and total area of abnormal fibrin clumps/clusters. Data from three independent experiments. (*I*) Western blotting shows Aβ42+fibrinogen treatment reduced SYP and PSD-95 levels in OHC. However, in the presence of lecanemab, the Aβ42/fibrinogen-mediated reduction in PSD-95 and SYP was minimized. (*J* and *K*) Quantification of *I*. Data from four experiments. Changes in synaptic markers were not due to cell death as determined by propidium iodide staining. (*L*) B-Aβ42 was incubated in NHP to form complexes with fibrinogen. Lecanemab, aducanumab, buffer, or control IgG was added after 1 h. Western blot analysis of immunoprecipitation shows that lecanemab dissociated preformed Aβ42/fibrinogen complexes while aducanumab did not. (*M*) Quantification of *L*. Data from three independent experiments. (*N*) Western blotting shows treatment with preformed Aβ42/fibrinogen complexes reduced SYP and PSD-95 levels in OHC, but lecanemab mitigated this effect. (*O* and *P*) Quantification of SYP and PSD-95. Data from three independent experiments. Vehicle constitutes PBS+DMSO. Comparisons among multiple groups were performed using one-way ANOVA followed by Newman–Keuls multiple comparison test. Data are presented as mean ± SEM. *****P* < 0.0001, ****P* < 0.001, ***P* < 0.01; ns, not significant.

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