Deficits in AD Mouse Models - Amyloid and Reverse

ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse

This copy is for your personal, non-commercial use only.
ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models


Alzheimer’s disease (AD) is associated with impaired clearance of β-amyloid (Aβ) from the brain, a process normally facilitated by apolipoprotein E (apoE). ApoE expression is transcriptionally induced through the action of the nuclear receptors peroxisome proliferator–activated receptor gamma and liver X receptors in coordination with retinoid X receptors (RXRs). Oral administration induced through the action of the nuclear receptors peroxisome proliferator–activated receptor gamma (PPARγ) and liver X receptors (LXRs) in coordination with retinoid X receptors (RXRs). Transcriptional activators, peroxisome proliferator–activated receptor gamma (PPARγ), and liver X receptors (LXRs) are normally facilitated by apolipoprotein E (apoE). ApoE expression is transcriptionally regulated by ligation of either member of PPARγ and LXRs to activate the ligand-activated nuclear receptors RXRs and RXR:RXR, inducing apoE, its lipid transporters ABCA1 and ABCG1, and the nuclear receptors themselves (7). Agonists of these receptors also act on macrophages and microglia to stimulate their conversion into “alternative” activation states (9) and promote phagocytosis (10). Chronic administration of LXR and PPARγ agonists reduces Aβ levels and improves cognitive function in mouse models of AD (10).

We reasoned that an RXR agonist would enhance normal Aβ clearance mechanisms by activating PPARγ:RXR and LXR:RXR, inducing apoE expression, facilitating Aβ clearance, and promoting microglial phagocytosis. Bexarotene (Targretin) is a highly selective, blood-brain barrier–permeant (fig. S3A), RXR agonist approved by the U.S. Food and Drug Administration (FDA) (11) with a favorable safety profile (12). Treatment of primary microglia or astrocytes with bexarotene stimulated the expression of apoE, ABCA1, and ABCG1 (fig. S1, A and B) and secretion of highly lipided HDL particles (fig. S1, C and D). Bexarotene treatment of primary microglia and astrocytes facilitated degradation of soluble Aβ42 (fig. S2, A and B) in a PPARγ-LXR (fig. S2, C and D)– and apoE (fig. S2, E and F)–dependent manner. The levels of Aβ peptides, insulin-degrading enzyme and neprilysin, were unchanged with bexarotene treatment (fig. S1, E and F).

Brain interstitial fluid (ISF) Aβ levels were monitored by hippocampal in vivo microdialysis (13) of 2-month-old APPswe/PS1ΔE9 (APP/PS1) mice. Bexarotene rapidly lowered ISF Aβ40 and Aβ42 levels within 6 hours of administration, with a 25% reduction by 24 hours (Fig. 1, A and B). One dose of bexarotene significantly decreased ISF Aβ40 and Aβ42 levels by 25% for more than 70 hours (Fig. 1D), with a return to baseline by 84 hours. The suppression of ISF Aβ42 was due to increased clearance, as the Aβ40 half-life was reduced from 1.4 to 0.7 hours (Fig. 1C). Bexarotene reduced murine Aβ levels in the C57Bl/6 mouse to a similar extent as in APP/PS1 mice; however, it had no effect on Aβ42 levels in apoE-null mice (Fig. 1E), demonstrating that the enhanced clearance of soluble ISF Aβ required apoE.

Fig. 1. ISF levels of Aβ decrease after bexarotene treatment. (A and B) ISF Aβ40 and Aβ42 levels were monitored by in vivo hippocampal microdialysis of 2-month-old APP/PS1 mice. Baseline Aβ levels were monitored for 6 hours, followed by daily orally administered bexarotene (100 mg kg−1 day−1) (Bex) or vehicle (Veh; water) for 3 days. Mice were coadministered compound E [20 mg kg−1 intraperitoneally (i.p.)] on day 3. (C) The elimination half-life of ISF Aβ40 was measured. In 2-month-old APP/PS1 mice, baseline ISF Aβ40 levels were sampled after administration of a single oral dose of bexarotene (100 mg kg−1). (D) ISF Aβ40 and Aβ42 were sampled every 2 to 6 hours for 4 days after treatment. (E) Baseline ISF Aβ levels of nontransgenic (C57Bl/6) and apoE knockout (KO) mice (2 months) with and without bexarotene treatment. ISF Aβ40 levels were measured between hours 7 and 12 after treatment; n = 5 mice per group (Student’s t test; mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001).

1Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA. 2Department of Neurology, Hope Center for Neurological Disorders, Knight Alzheimer’s Disease Research Center, Washington University School of Medicine, St. Louis, MO 63110, USA. 3Emotional Brain Institute, Nathan Kline Institute for Psychiatric Research and the New York University School of Medicine, Orangeburg, NY 10962, USA. 4Center of Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

*To whom correspondence should be addressed. E-mail: gei2@case.edu
We observed the rapid removal of both diffuse and compact Aβ plaques in the cortex and hippocampus of APP/PS1 mice after acute treatment with bexarotene (Fig. 2). We orally administered bexarotene or vehicle daily to 6-month-old APP/PS1 mice for 3, 7, or 14 days. We observed the progressively enhanced expression of apoE, ABCA1, and ABCG1 and elevated HDL levels in both the hippocampus and cortex of bexarotene-treated mice (Fig. S3, B and C). There was a sustained 30% reduction in soluble Aβ levels throughout the 14-day treatment period (Fig. 2A). Insoluble Aβ levels were reduced by 40% after 72 hours and progressively decreased over the subsequent 14 days (Fig. 2A). Total (Fig. 2, B and C) and thioflavin-S+ Aβ plaques (Fig. 2, E and F) were reduced by ~75% after 14 days of bexarotene treatment. Furthermore, we observed abundant Aβ-laden microglia after 3 days of bexarotene treatment, suggesting their involvement in the phagocytic removal of Aβ deposits (Fig. 2D).

To assess whether bexarotene could decrease Aβ burden in older animals with greater plaque deposition, we treated 11-month APP/PS1 mice with bexarotene for 7 days and found significantly reduced levels of soluble and insoluble Aβ40 and Aβ42 (Fig. S4C), a 50% reduction in plaque number (Fig. S4, D and E), and a concurrent increase in expression of apoE, the cholesterol transporters, and HDL levels (fig. S4, A and B). Thus, the efficacy of acute bexarotene treatment is evident in both early and later stages of pathogenesis in this mouse model.

We also tested the effect of chronic bexarotene treatment (3 months, daily) of APP/PS1 mice starting from 6 months of age. We found elevated levels of apoE, ABCA1, ABCG1, and HDL (fig. S5, A and B). Bexarotene reduced soluble Aβ levels by ~30%, consistent with its ability to enhance apoE-dependent Aβ proteolysis (fig. S6C). However, amyloid plaque burden was unchanged (fig. S5, D to G).

To evaluate the robustness of the effect of bexarotene, we treated an aggressive model of amyloidosis, the APPPS1-21 mouse (14), which possesses high levels of deposited Aβ7 to 8 months of age. APPPS1-21 mice treated for 20 days with bexarotene exhibited a reduced level of soluble and insoluble Aβ peptides (fig. S6C) and a 35% decrease in the number of thioflavin S+ plaques (fig. S6, D and E). Bexarotene treatment enhanced the expression of ABCA1, ABCG1, apoE, and its lipidated forms (fig. S6, A and B).

There is persuasive evidence that the cognitive and behavioral deficits characteristic of AD arise, in part, from impaired synaptic function due to soluble forms of Aβ. Bexarotene treatment rapidly restored cognition and memory, as assessed by contextual fear conditioning in APP/PS1 mice treated for 7 days at both early (6 months) and later (11 months) stages of plaque pathogenesis. Similarly, chronic treatment of 6-month-old APP/PS1 mice for 90 days (analyzed at 9 months of age) (Fig. 3, A to C) showed drug-induced behavioral improvements in the contextual fear conditioning task. Additionally, APP/PS1 mice treated for 90 days and APPPS1-21 mice treated for 20 days exhibited improved hippocampal function after bexarotene treatment, as assessed by Morris water maze performance (Fig. 3, D and F), as well as in the contextual fear conditioning assay (Fig. 3E).

Nest construction is an affiliative, social behavior that becomes progressively impaired in Tg2576

---

**Fig. 2.** Aβ levels and plaque burden are reduced by bexarotene treatment. APP/PS1 or nontransgenic (NonTg) mice (6 months) orally gavaged for 3, 7, and 14 days with bexarotene (100 mg kg\(^{-1}\) day\(^{-1}\)) or vehicle (water). Soluble and insoluble Aβ40 and Aβ42 levels were measured by enzyme-linked immunosorbent assay. (A) Fold changes based on vehicle: 7.0445 ng/mg protein and 36.8 ng/mg protein of soluble Aβ40 and Aβ42, respectively. (B and C) Total (Fig. 2, B and C) and thioflavin-S+ Aβ plaques (Fig. 2, E and F) were reduced by ~75% after 14 days of bexarotene treatment. (D) Representative image of microglia in the cortex of a 6-month APP/PS1 mouse treated for 3 days with bexarotene (red: 6E10; green: Iba1; blue: DAPI). Scale bar: 10 μm.
mice (15). After just 72 hours of bexarotene treatment, nest construction behavior was restored in Tg2576 mice (Fig. 3G).

Finally, we explored whether bexarotene could rescue olfactory sensory impairments, (16), which are highly correlated with Aβ deposition in Tg2576 mice (17). Bexarotene treatment improved odor habituation behavior after 9 days of drug treatment in Tg2576 mice 12 to 14 months of age (Fig. 3H).

The improved behaviors observed in bexarotene–treated mice suggest global improvements of neural network function. Soluble Aβ interferes with synaptic function that subserves higher-order neural network information processing (3). Piriform cortex (PCX) circuit function is critical to odor-guided behaviors, and its disruption is implicated in impaired olfactory perception in both humans with AD and in Tg2576 mice (18). Therefore, we evaluated odor-evoked PCX local field potentials (LFPs) as a behaviorally relevant synaptic readout of neural circuit status. Odor-evoked high-frequency gamma-band oscillations (35 to 75 Hz) and beta-band oscillations (15 to 35 Hz), reflecting local circuit interactions and interregional network activity, respectively, are considered critical for normal olfactory function (18, 19). Tg2576 mice (12 to 14 months) treated with vehicle exhibited significantly less odor-evoked beta- and gamma-band LFP power compared to drug-treated nontransgenic mice (Fig. 4, A and B), which was restored by 3 days of bexarotene treatment. Odor habituation after bexarotene treatment was improved in these same mice (fig. S7, B and C), indicating a rapid drug-dependent normalization of local and regional circuit function in the primary olfactory pathway.

RXR activation stimulates the normal physiological processes through which Aβ is cleared from the brain. The dependence of soluble Aβ clearance on apoE validates the mechanistic linkage between the principal genetic risk factor for AD and the cognitive impairment that characterizes the disease (5, 6). Bexarotene acts rapidly to facilitate the apoE-dependent clearance of soluble forms of Aβ, accounting for the extremely rapid change in ISF Aβ metabolism. Bexarotene-mediated behavioral improvements were correlated with a reduction in soluble Aβ peptide levels of ~30%. These observations are consistent with previous observations that learning and memory
can be improved through reducing brain-soluble Aβ levels, either upon the administration of β- or γ-secretase inhibitors (20, 21) or provision of antibodies against Aβ (22). However, the behavioral improvements were poorly correlated with the microglial-mediated removal of insoluble, deposited forms of Aβ. The dual actions of the nuclear receptors resulting in the enhanced expression and lipolysis of apoE and modulation of the microglial-mediated immune response are consistent with recent genetic association analyses implicating them in the etiology of AD (23–25). The ability of bexarotene to rapidly reverse a broad range of deficits suggests that RXR agonists may be of therapeutic utility in the treatment of AD and its antecedent phases.

References and Notes

Acknowledgments: We thank Dr. Mangelsdorf for discussions and M. Pendergast, G. Casadesus, and I. Nagle for technical assistance. This work was supported by the Blanchette Hooker Rockefeller Foundation, Thome Foundation, Rody and Taft Funds for Alzheimer’s Research, Foundation, American Health Assistance Foundation, Cure Alzheimer’s Fund, Coins for Alzheimer’s Research Trust, and the National Institute on Aging (NIA) (grant AG030482-03S1 to G.E.L.); National Institute on Deafness and Other Communication Disorders (grant DC003906, RO1-AG037693 to D.A.W.); NIA (grants KO1 AG025924 and P50-AG05681), Shmerler family, and the Charles F. and Joanne Knight Alzheimer’s Disease Research Center at Washington University (to J.R.C.); and Marian S. Ware Alzheimer Program to K.R.B.). All raw data are archived in Igel-sever1 for authorized users. P.E.C. and G.E.L. hold U.S. Provisional Patent Application no. 61/224,709 regarding bexarotene as a potential therapeutic for Alzheimer’s disease and are founding scientists of ReXceptor, Inc., which has licensing options from Case Western Reserve University on the use of bexarotene in the treatment of Alzheimer’s disease.

Supporting Online Material
www.sciencemag.org/cgi/content/full/science.1217697/DC1
Materials and Methods
Figs. S1 to S7
References (26–31)
9 December 2011; accepted 20 January 2012
Published online 9 February 2012; 10.1126/science.1217697

Long-Range-Projecting GABAergic Neurons Modulate Inhibition in Hippocampus and Entorhinal Cortex

Sarah Melzer,1* Magdalena Michael,1* Antonio Caputi,1* Marina Eliava,1 Elke C. Fuchs,1 Miles A. Whittington,2 Hannah Monyer1†

The hippocampus and entorhinal cortex play a pivotal role in spatial learning and memory. The two forebrain regions are highly interconnected via excitatory pathways. Using optogenetic tools, we identified and characterized long-range γ-aminobutyric acid–releasing (GABAergic) neurons that provide a bidirectional hippocampal-entorhinal inhibitory connectivity and preferentially target GABAergic interneurons. Activation of long-range GABAergic axons enhances sub- and suprathermal rhythm theta activity of postsynaptic neurons in the target areas.

The excitatory projections connecting the hippocampus and entorhinal cortex (1) account for the functional interdependence of these two brain regions (2–4). Excitatory neurons in the hippocampus and entorhinal cortex are under control of local γ-aminobutyric acid–releasing (GABAergic) interneurons (5, 6). Some GABAergic neurons also project long distance. For example, long-range–projecting GABAergic cells connect hippocampus with medial septum (7–9) and other extra-hippocampal brain areas (10, 11), suggesting that interregional GABAergic connectivity might be less rare than was previously assumed (12).

To test for the presence of hippocampal GABAergic neurons projecting to the medial entorhinal cortex (MEC), we injected the retrograde tracer fluorogold (FG) into the MEC of wild-type mice (fig. S1). In addition to the expected labeling of numerous excitatory cells, we found FG+ neurons in stratum oriens and stratum radiatum of CA1 and in the hilus of the dentate gyrus (DG), indicating retrogradely labeled GABAergic cells. We detected FG-labeled cells coexpressing somatostatin (SOM) in stratum oriens of CA1 (23 cells, nine mice) and also in the hilus of the DG (14 cells, nine mice) (Fig. 1, A and B).

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: h.monyer@dkfz-heidelberg.de

1Department of Clinical Neurobiology of the Medical Faculty of Heidelberg University and German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany.
2Institute of Neurosciences, The Medical School, Newcastle University, Framlington Place, Newcastle, NE2 4HH, UK.