ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models

Paige E. Cramer,1 John R. Cirrito,2 Daniel W. Wesson,1,3 C. Y. Daniel Lee,1 J. Colleen Karlo,1 Adriana E. Zinn,1 Brad T. Casali,1 Jessica L. Restivo,2 Whitney D. Goebel,2 Michael J. James,4 Kurt R. Brunden,4 Donald A. Wilson,3 Gary E. Landreth1*

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: gel2@case.edu

Alzheimer’s disease is associated with impaired clearance of β-amyloid 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 (PPARγ) and liver X receptors (LXRs) in coordination with retinoic X receptors (RXR). Oral administration of the RXR agonist, bexarotene, to a murine model of Alzheimer’s disease resulted in enhanced clearance of soluble Aβ within hours in an apoE-dependent manner. Aβ plaque area was reduced >50% within just 72 hours. Furthermore, bexarotene stimulated the rapid reversal of cognitive, social, and olfactory deficits and improved neural circuit function. Thus, RXR activation stimulates physiological Aβ clearance mechanisms resulting in the very rapid reversal of a broad range of Aβ-induced deficits.

The most common form of Alzheimer’s disease (AD) occurs sporadically late in life and is typified by deposition of β-amyloid (Aβ) within the brain (1). Individuals with late-onset AD produce Aβ peptides at normal levels but have an impaired ability to clear them from the brain (2). Elevated levels of Aβ are associated with perturbations of synaptic function and neural network activity that probably underlie the cognitive deficits in AD (3). Moreover, Aβ accumulation leads to its deposition into plaques and is thought to drive a pathologic cascade which ultimately leads to neuronal death. The most influential genetic risk factor for sporadic AD is allelic variation in the apolipoprotein E (APOE) gene. Possession of an APOE4 allele dramatically increases disease risk (4). ApoE acts normally to scaffold the formation of high density lipoprotein (HDL) particles which promote the proteolytic degradation of soluble forms of Aβ (5, 6).

The expression of apoE is transcriptionally regulated by the ligand-activated nuclear receptors, peroxisome proliferator-activated receptor gamma (PPARγ) and liver X receptors (LXRs) (7), which form obligate heterodimers with retinoid X receptors (RXRs). Transcriptional activity is regulated by ligation of either member of the pair (8). PPARγ:RXR and LXR:RXR act in a feed-forward manner to induce the expression of 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 reduce Aβ levels and improve cognitive function in mouse models of AD (10).

We reasoned that an RXR agonist would enhance normal Aβ clearance mechanisms by activating PPAR: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), FDA-approved, RXR agonist (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-B), and secretion of highly lipiddated HDL particles (fig. S1 C-D). Bexarotene treatment of primary microglia and astrocytes facilitated degradation of soluble Aβ42 (fig. S2 A-B), in a PPARγ, LXR (fig. S2 C-D), and apoE (fig. S2 E-F) dependent manner. The levels of Aβ proteases, insulin degrading enzyme and nephrilysin, were unchanged with bexarotene treatment (fig. S1 E-F).

Brain interstitial fluid (ISF) Aβ levels were monitored by hippocampal in vivo microdialysis (13) of two 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, B). One dose of bexarotene significantly decreased ISF Aβ_{40} and Aβ_{42} levels by 25% for over 70 hrs (Fig. 1D), with a return to baseline by 84 hours. The suppression of ISF Aβ was due to increased clearance, as the Aβ_{40} half-life was reduced from 1.4 to 0.7 hours (Fig. 1C). Bexarotene reduced murine Aβ levels in the C57Bl/6 mice to a similar extent as in APP/PS1 mice; however, it had no effect on Aβ levels in apoe-null mice (Fig. 1E), demonstrating that the enhanced clearance of soluble ISF Aβ required apoe.

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, ABCG1 and elevated HDL levels in both the hippocampus and cortex of bexarotene-treated mice (Fig. S3 B-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-C) and thioflavin-S+ Aβ plaques (Fig. 2, E-F) were reduced by approximately 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 was able to 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 Aβ_{40} and Aβ_{42} (fig. S4C), a 50% reduction in plaque number (fig. S4 D-E), and a concurrent increase in expression of apoe, the cholesterol transporters and HDL levels (fig. S4 A-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-B). Bexarotene reduced soluble Aβ levels by approximately 30%, consistent with its ability to enhance apoe-dependent Aβ proteolysis (fig. S6C). However, amyloid plaque burden was unchanged (fig. S5 D-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 Aβ at 7-8 months of age. APPPS1-21 mice treated for 20 days with bexarotene exhibited a reduction of soluble and insoluble Aβ peptides (fig. S6C) and a 35% decrease in the number of thioflavin S+ plaques (fig. S6 D-E). Bexarotene treatment enhanced the expression of ABCA1, ABCG1, apoE and its lipidated forms (fig. S6 A-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 mo) and later (11 mo) stages of plaque pathogenesis. Similarly, chronic treatment of 6 month old APP/PS1 mice treated for 90 days (analyzed at 9 mo of age) (Fig. 3 A-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 following bexarotene treatment, as assessed by Morris water maze performance (Fig. 3 D and F), as well as in the as contextual fear conditioning assay (Fig. 3E).

Nest construction is an affiliative, social behavior that becomes progressively impaired in Tg2576 mice (15). Following 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 following 9 days of drug treatment in Tg2576 mice 12-14 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 read-out of neural circuit status. Odor-evoked high-frequency gamma band oscillations (35-75Hz) and beta band oscillations (15-35Hz), reflecting local circuit interactions and inter-regional network activity, respectively, are considered critical for normal olfactory function (18, 19). Tg2576 mice (12-14 mo) treated with vehicle exhibited significantly less odor-evoked beta and gamma band LFP power compared to drug-treated non-transgenic mice (Fig. 4 A-B), which was restored by 3 days of bexarotene treatment. Odor habituation following bexarotene treatment was improved in these same mice (fig. S7 B-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 (6, 20). 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 reduction in soluble Aβ peptide levels of approximately 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 beta or gamma secretase inhibitors (21, 22) or provision of anti-Aβ antibodies (23). 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 lipidaion of apoE and modulation of the microglial-mediated immune response are consistent with recent genetic association analyses implicating them in the etiology of AD (24–26). 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
8. P. Lefebvre, Y. Benomar, B. Staels, Trends Endocrinol Metab, (2010).
16. C. Murphy, Physiol Behav 66, 177 (1999).

Acknowledgments: We thank Dr. Mangelsdorf for discussions and M. Pendergast, G. Casadesus and I. Nagle for technical assistance. This work was supported by the Blancheet Hooker Rockefeller Foundation, Thome Foundation, Roby and Taft Funds for Alzheimer's Research and the NIA, AG030482-03S1 to GEL; NIDCD, DC003906, RO1-AG005681, Shmerler family, the Charles F. and Joanne Knight ADRC at Washington University to J.R.C.; and Marian S. Ware Alzheimer Program to KRB. All data is archived on $gel-server1. PEC and GEL hold a US 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 CWRU 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 (27–32)

9 December 2011; accepted 20 January 2012
Published online 9 February 2012; 10.1126/science.1217697
APP/PS1 mice, baseline ISF Aβ levels were sampled following administration of a single oral dose of bexarotene (100mg/kg). ISF Aβx-40 and Aβx-42 were sampled every 2-6 hours for 4 days after treatment (D). Baseline ISF Aβ levels of non-transgenic (C57Bl/6) and apoE knockout mice (2 mo) with and without bexarotene treatment (E). ISF Aβx-40 levels were measured between hours 7 and 12 after treatment; n=5/group. (Student’s t test. mean±SEM, *p<0.05, **p<0.01, ***p<0.001).

Fig. 2. Aβ levels and plaque burden are reduced by bexarotene treatment. APP/PS1 or non-transgenic (NonTg) mice (6 mo) orally gavaged for 3, 7 and 14 days with bexarotene (100 mg/kg/day) or vehicle (water). Soluble and insoluble Aβ40 and Aβ42 levels measured by ELISA. Fold changes based on vehicle: 7.0445 ng/mg protein and 14.529 ng/mg protein soluble Aβ40 and Aβ42, respectively and 30.349 ng/mg protein and 36.8 ng/mg protein insoluble Aβ40 and Aβ42, respectively (A). Representative cortex and hippocampus sections (B, E) of vehicle and 14 day bexarotene treated mice stained with the anti-Aβ antibody, 6E10 (B) or thioflavin S (E) are shown and plaque levels quantified (C, F); n≥5 animals/group (Student’s t test. mean±SEM *p<0.05, **p<0.01, ***p<0.001 Scale bar: cortex 100µm, hippocampus 200 µm). Representative image of microglia in the cortex of a 6 mo APP/PS1 mouse treated for 3 days with bexarotene (D) (Red:6E10, Green:Iba1, Blue:DAPI, Scale bar: 10µm).

Fig. 3. Restoration of memory and cognition with bexarotene treatment. Contextual fear-learning assayed in 6 (A) and 11 mo old (B) APP/PS1 mice treated for 7 days, or in 9 mo old, APP/PS1 mice treated for 90 days (C) with vehicle or bexarotene. APPPS1-21 mice 7-8 mo of age were treated for 20 days and performance evaluated (E). Percent time frozen was recorded in the 5 min test trial. Spatial memory was assessed using the Morris water maze (D, F). Time spent in the NW quadrant in the retention probe of 7-8 mo old, 20 day-treated APPPS1-21 (D) and 9 mo old, 90 day-treated APP/PS1 mice (F) with vehicle or bexarotene (Bex) 100mg/kg/day. (Non-transgenic littermates were controls (NonTg), n=7-14/group, Student’s t test. mean±SEM *p<0.05, **p<0.01). Nest construction was quantified in 12-14 mo NonTg and Tg2576 mice (G). Baseline data were obtained on day 0, following daily drug treatment and addition of paper towels in clean cages. (2-tailed t test *p<0.05, **p<0.01). Odor habituation behavior in 12-14 mo Tg2576 mice tested before (baseline) and after 9 days of bexarotene treatment (H) n=5/group (2-tailed t-test; mean±SEM **p<0.01, ***p<0.001 Tg2576 baseline vs. Tg2576 Bex).

Fig. 4. Rescue of cortical network activity with bexarotene. LFP recordings of Tg2576 or non-transgenic (NonTg) mice (12-14mo) gavaged with bexarotene (Bex) (100mg/kg) or vehicle (H2O) for 3 days following implantation of electrodes into PCX. PCX LFPs in response to the odor ethyl valerate in an awake non-transgenic, bexarotene treated mouse. 15-35Hz beta and 35-75Hz gamma band power traces (2nd-order band pass) (A). PCX odor-evoked response magnitudes (2sec odor/2sec pre-odor) (B). (n=5 mice/group, 4 odor presentations/mouse. *p<0.05, **p<0.01, ***p<0.001, mean±SEM 2-tailed t-tests of mean odor-evoked magnitudes within LFP bins).