Comment on “ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models”

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Cramer et al. (Reports, 23 March 2012, p. 1503; published online 9 February 2012) reported that bexarotene rapidly reduces β-amyloid (Aβ) levels and plaque burden in two mouse models of Aβ deposition in Alzheimer’s disease (AD). We now report that, although bexarotene reduces soluble Aβ40 levels in one of the mouse models, the drug has no impact on plaque burden in three strains that exhibit Aβ amyloidosis.

Alzheimer’s disease (AD), the major cause of adult-onset dementia, is characterized by progressive memory loss and severe cognitive decline that are associated with cerebral deposition of β-amyloid (Aβ) peptides. Familial, autosomal dominant AD (FAD) is caused by expression of mutant variants of Aβ precursor proteins (APP) and presenilins (PS), and expression of these mutant genes in mice leads to cerebral deposition of Aβ peptides (1). Cramer et al. (2) reported that bexarotene (Targetretin), a retinoic X receptor (RXR) agonist approved by the U.S. Food and Drug Administration (FDA), rapidly reduces Aβ levels in the interstitial fluid, reduces amyloid plaque burden, and rescues behavioral deficits in transgenic mouse models of Aβ amyloidosis. For example, 6-month-old mice expressing FAD-linked APPsw and PS1ΔE9 poly peptides [APP/PS1 mice (3)] that received daily oral doses of Targetretin exhibited a reduction of Aβ plaques by ~75% within 7 days and significantly reduced levels of soluble and insoluble Aβ peptides that accompanied increases in brain levels of apoE, ABCA1, ABCG1, encoded by RXR-target genes, and elevated levels of highly lipidated HDL. Similarly, Cramer et al. (2) reported that 8-month-old mice expressing APPsw and PS1L166P poly peptides [APPPS1-21 mice (4)], treated with Targetretin for 20 days showed lowered Aβ levels and amyloid plaques. Finally, Cramer et al. (2) reported the presence of Aβ-laden microglia in mice treated with Targetretin for 3 days, although a similar analysis was not reported for vehicle (H2O)-treated animals.

In view of the important implications of these findings for the development of novel AD therapeutics, we have attempted to replicate these findings. We noted that Cramer et al. (2) used a limited number of mice (N = 5 for APP/PS1 mice), and of mixed gender, the latter a confound in the author’s interpretation of results, because female APP/PS1 mice exhibit accelerated amyloid deposition, elevated Aβ deposition, and elevated amyloid burden, particularly at 6 months of age, compared with their male counterparts (3).

We performed studies on three mouse models of Aβ amyloidogenesis. In the first, cohorts of 6-month-old male APP/PS1 mice (3) [the same strain used by Cramer et al. (2)], were treated orally with 100 mg per kg of weight (mg/kg) of Targetretin or vehicle (6.6% dimethyl sulfoxide; 4% ethanol; 89.6% sunflower oil) for seven consecutive days. Fixed hemibrains were sectioned and brain levels of apoE, ABCA1, ABCG1, encoded by RXR-target genes, and elevated levels of highly lipidated HDL. Similarly, Cramer et al. (2) reported that 8-month-old mice expressing APPsw and PS1L166P poly peptides [APPPS1-21 mice (4)], treated with Targetretin for 20 days showed lowered Aβ levels and amyloid plaques. Finally, Cramer et al. (2) reported the presence of Aβ-laden microglia in mice treated with Targetretin for 3 days, although a similar analysis was not reported for vehicle (H2O)-treated animals.

In the second, cohorts of 9-month-old male APPPS1-21 mice (4) with 100 mg/kg Targetretin suspended in H2O or with H2O as the vehicle. This strain was also used by Cramer and colleagues (2) and exhibits robust Aβ deposition by 8 months of age. Sections were stained with a polyclonal antibody to Aβ [CN3 (9)] and Fig. 2, H and I, are representative Aβ-labeled sections from individual animals treated with H2O or Targetretin, respectively. Analysis of amyloid plaque load in four male mice per group failed to reveal a significant difference between H2O or Targetretin-treated animals (Fig. 2), and Meso Scale Discovery (MSD) analysis using the human 6E10 Aβ triplex assay revealed a medium effect size for soluble Aβ species and a small effect size for insoluble Aβ species (2K). Finally, we demonstrated an ~1.5-fold elevation in ABCA1 levels in the brains of Targetretin-treated animals compared with the vehicle cohort (Fig. 2, 2F).

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Fig. 1. Effects of Targretin on amyloid plaque burden and Aβ levels in APP/PS1 mice. Representative images of hemibrain sections from 6-month-old APP/PS1 mice treated with vehicle (A) or 100 mg/kg Targretin (D) that were immunostained with 3D6 antibody. Scale bar, 500 μm. (B) and (E) Representative confocal z-stack projection images of Iba1+ microglia (green) surrounding 3D6+ amyloid plaque deposits (red) in sections from vehicle-treated (B) or Targretin-treated (E) animals. (C and F) Overlay and orthogonal views (XY/XZ sectional) of images shown in (B) and (E), respectively. Arrows point to the intracellular 3D6 immunoreactivity in microglia of both sample groups. These results suggest that microglial phagocytosis of Aβ is not enhanced in Targretin-treated animals. Scale bar, 25 μm. Sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). (G), (H), (I), and (K) represent mean ± SEM; N = 7 mice per group. 

References and Notes


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Fig. 2. Effects of Targretin on amyloid plaque burden and Aβ levels in 5XFAD and APPPS1-21 mice. (A to G) Representative images of hemibrain sections from 3- to 4-month old 5XFAD mice treated with vehicle (A) or 100 mg/kg Targretin (B) for 7 days that were immunostained with 3D6 antibody. Scale bar, 500 μm. (C and D) Histograms show the amyloid plaque area fraction and the total number of plaques in cortex (CX) or hippocampus (HP), respectively. Total area examined in vehicle- versus Targretin-treated groups are comparable (117,841.3 ± 3303.082 μm² for vehicle group versus 107,086.3 ± 6802.094 μm² for Targretin group, in cortex with P = 0.17; 67,039.59 ± 4030.787 μm² for vehicle group versus 70,535.3 ± 2843.477 μm² for Targretin group, in hippocampus with P = 0.486). (E) Histogram shows fold changes in soluble (s) Aβ40 (*P = 0.008; Cohen’s d: 1.25), insoluble (ins) Aβ40 (#P = 0.292; Cohen’s d: 0.46), soluble Aβ42 ($P = 0.102; Cohen’s d: 0.73), and insoluble Aβ42 (%P = 0.363; Cohen’s d: 0.396) levels after normalization against total protein content. (F) Representative Western blots of detergent-soluble total protein lysates (60 μg per lane) from hemibrains of vehicle- or Targretin-treated 5XFAD animals using antibodies to ABCA1 or β-III tubulin. (G) Quantification of ABCA1 band intensity normalized against β-III tubulin levels (*P = 0.004). Data in (C), (D), (E) and (G) represent mean ± SEM; N = 11 mice per group. (H to M) Representative images of hemibrain sections from 9-month-old APPPS1-21 mice treated with vehicle (H2O) (H) or 100 mg/kg Targretin (I) for 26 days that were immunostained with Aβ-specific CN3 antibody. Scale bar, 500 μm. (J) Histogram shows the percentage of amyloid plaque load assessed in neocortex on random sets of every 12th systematically sampled 40-μm-thick sections, analyzed by area fraction technique estimated with the aid of Stereologer software (mean ± SEM; N = 4 mice per group). (K) Histogram shows fold changes in soluble (s) Aβ40 (*P = 0.269; Cohen’s d: 0.32), insoluble (ins) Aβ40 (#P = 0.49; Cohen’s d: 0.013), soluble Aβ42 ($P = 0.182; Cohen’s d: 0.325), and insoluble Aβ42 (%P = 0.495; Cohen’s d: 0.008) levels in vehicle- versus Targretin-treated APPPS1-21 mice, respectively. (L) Representative Western blots of detergent-soluble total protein lysates (60 μg per lane) from hemibrains of vehicle- or Targretin-treated APPPS1-21 mice using antibodies to ABCA1 or β-III tubulin. (M) Quantification of ABCA1 band intensity normalized against β-III tubulin levels (*P = 0.04). Data in (K) and (M) represent mean ± SEM; N = 3 mice per group.)
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