Reducing Available Soluble β-Amyloid Prevents Progression of Cerebral Amyloid Angiopathy in Transgenic Mice

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Abstract
Cerebral amyloid angiopathy (CAA), the accumulation of β-amyloid (Aβ) in the walls of leptomeningeal and cortical blood vessels of the brain, is a major cause of intracerebral hemorrhage and cognitive impairment and is commonly associated with Alzheimer disease. The progression of CAA, as measured in transgenic mice by longitudinal imaging with multiphoton microscopy, occurs in a predictable linear manner. The dynamics of Aβ deposition in and clearance from vascular walls and their relationship to the concentration of Aβ in the brain are poorly understood. We manipulated Aβ levels in the brain using 2 approaches: peripheral clearance via administration of the amyloid binding “peripheral sink” protein gelsolin and direct inhibition of its formation via administration of LY-411575, a small-molecule γ-secretase inhibitor. We found that gelsolin and LY-411575 both reduced the rate of CAA progression in Tg2576 mice from untreated rates of 0.58% ± 0.15% and 0.52% ± 0.09% to 0.11% ± 0.18% (p = 0.04) and −0.17% ± 0.09% (p < 0.001) of affected vessel per day, respectively, in the absence of an immune response. The progression of CAA was also halted when gelsolin was combined with LY-411575 (−0.004% ± 0.10%, p < 0.003). These data suggest that CAA progression can be prevented with non-immune approaches that may reduce the availability of soluble Aβ but without evidence of substantial amyloid clearance from vessels.

Key Words: Alzheimer, Amyloid, Cerebral amyloid angiopathy, Cerebrovasculature, Gamma secretase, Gelsolin, Imaging, Multiphoton.

INTRODUCTION
Cerebral amyloid angiopathy (CAA) is an age-related disease found in Alzheimer disease (AD) patients and in the normal aging population (1). Like the senile plaques of AD, the cerebrovascular deposits in CAA are primarily composed of the 38–42–amino acid β-amyloid (Aβ) peptide, although they are dominated by the shorter peptide fragments (2). The pathologic manifestations of CAA include vascular smooth muscle cell degeneration and hemorrhagic and ischemic stroke (3). The CAA-related hemorraghes are not readily preventable and represent a serious and debilitating outcome in affected patients. Other adverse events associated with CAA have been identified in clinical trials using immunotherapy for AD that have resulted in a range of clinical, neuroimaging, and neuropathologic abnormalities apparently related to vascular amyloid (4–6). In part because of the adverse effects associated with anti-amyloid immunotherapy, much attention has focused on non-immune methods. Indeed, several agents that are unrelated to antibodies or immune-based therapies have been effective at altering Aβ levels in the brain, plasma, and cerebrospinal fluid (CSF). One approach is to sequester plasma Aβ, leading to an alteration in the dynamic equilibrium of brain and peripheral Aβ (7). This “peripheral sink” approach does not require antibodies to enter the brain but rather the egress of brain Aβ to the periphery that in turn enhances clearance of brain deposits. Testing the effectiveness of the peripheral sink mechanism necessitates non–antibody-mediated sequestration of Aβ in the periphery to exclude neuroinflammatory mechanisms. Peripheral sequestration has been demonstrated using the 89-kd protein gelsolin, which has a high affinity for Aβ and that can reduce brain Aβ by binding plasma Aβ without the associated neuroinflammation (8).

Another non–immune-based method to influence Aβ concentration in the brain is by inhibiting Aβ production. The Aβ peptide is generated by sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. Small-molecule γ-secretase inhibitors have been developed to reduce Aβ production in the brain (9) and have been successfully used in AD patients and in transgenic mice to reduce plasma, CSF, and brain Aβ (10, 11). A well-characterized and potent γ-secretase inhibitor is N2-((2S)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl)-N1-((?S)-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl)-alaninamide (LY-411575) (12). Although LY-411575 has known side effects that limit its...
usefulness as a human therapeutic, it is very effective in reducing Aβ production as a proof-of-concept drug in experimental models. Previous studies in mice have shown dramatic effects on soluble amyloid levels and prevention of amyloid deposition but little effect on existing neuritic plaques of APP/PS1 transgenic mice (13). The ability of either LY-411575 or gelsolin to prevent or clear vascular Aβ is unknown.

Our previous work has characterized the transgenic mouse models that overexpress APP and have established that CAA progresses in a predictable linear manner over a range of ages (14–16). A robust system for visualizing the progression of CAA in vivo has also been developed where the deposition of Aβ in leptomeningeal vessels of transgenic mice can be imaged through a cranial window using multiphoton microscopy (15–17). This powerful approach allows longitudinal imaging of individually identified vessels in the brain, minimizing the number of animals required for statistical power. Using this system, we have previously demonstrated low levels of CAA clearance with direct exposure to an anti-amyloid monoclonal antibody (15). In the current study, we assessed whether a peripheral sink of Aβ (gelsolin) to enhance clearance and a potent inhibitor of amyloid production (LY-411575) can influence the progression of CAA measured with in vivo multiphoton microscopy in Tg2576 mice. We observed that both gelsolin and LY-411575 were able to reduce the rate of CAA progression alone, although without additive or synergistic effects of combination treatment. These results suggest that vascular amyloid deposition can be altered dynamically, providing confidence that a clinically relevant treatment for CAA can be developed.

MATERIALS AND METHODS

Animals and Drug Treatments

Male and female Tg2576 mice (18) aged 11 to 12 months were placed into 4 treatment groups. One experimental group received the Aβ-binding protein gelsolin (extracted from bovine plasma; 0.6 mg/kg in 10% Cremophor EL in PBS) via intraperitoneal injection every 2 days for 2 weeks (8). A second experimental group received the γ-secretase inhibitor. Because the therapeutic window for this compound does not seem to be affected by oral or peripheral administration (12), LY-411575 (15 mg/kg in 10% dimethylsulfoxide and 5% Cremophor EL in PBS) was administered daily via intraperitoneal injection for 3 weeks. A third group received a combined treatment of gelsolin and LY-411575 for 3 weeks. The last group was treated with vehicle alone (10% Cremophor in PBS) and used as a control. Gelsolin was purchased from Sigma (St. Louis, MO), and LY-411575 was a gift from Dr. Abdul H. Fauq, synthesized as described (19) and tested for potency using cell-based assays (20). All animal studies were in compliance with the Massachusetts General Hospital Animal Care and Use Committee and National Institutes of Health guidelines.

Multiphoton Microscopy and Image Analysis

All mice had permanent cranial windows placed for serial in vivo imaging via multiphoton microscopy, as previously described (15–17). Briefly, mice were anesthetized using isoflurane and 7-mm craniotomies were performed. The dura was left intact, and glass coverslips were attached using dental cement, allowing long-term visualization of the brain vasculature. Mice were imaged immediately after surgery (Day 0) while under anesthesia and reimaged at Day 7, Day 14, and Day 21. Then Aβ was visualized using the Congo red derivative methoxy-X04, administered via intraperitoneal injection 1 day before each imaging session (21). An angiogram was also obtained using Texas Red Dextran (0.2 mL; 12.5 mg/mL; 70,000 MW; Invitrogen, Eugene, OR), administered intravenously. This allowed consistent alignment of vessels during the imaging period.

In vitro imaging of CAA progression was performed as previously described (15–17) using a BioRad 1024 multiphoton microscope (Hercules, CA), with a Ti:Sapphire laser (Mai Tai; Spectra Physics, Mountain View, CA) used at 800 nm for excitation. External photodetectors (Hamamatsu Photonics, Hamamatsu City, Japan) collected blue fluorescent signal from bound methoxy-X04. Multiple fields (615 × 615 μm²) from both left and right hemispheres were imaged along with leptomeningeal cerebral arteries using a 20× objective (numerical aperture, 0.95; Olympus UPlanFl; Olympus, Melville, NY) to a depth of approximately 200 μm in 5-μm steps. Maximum intensity projections from z-stacks were generated using ImageJ software.

Quantitative analysis of CAA progression in individually identified vessels was performed using ImageJ and Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA), as previously described (15–17). Areas of CAA-affected vessels were outlined and manually thresholded to distinguish CAA from non-CAA regions. Pixel counts based on 6 or more contiguous pixels in CAA regions were determined in addition to total pixel counts in the entire outlined vessel segment. The CAA burden was calculated as the percentage of vessel area affected by CAA. Total vessel areas were determined from initial (Day 0) imaging sessions.

Immunohistochemistry

Postmortem 30-μm (floating) brain sections of untreated and gelsolin-treated Tg2576 mice were immunostained using antibodies specific for astrocytes and microglia. Sections were permeabilized using 0.1% Triton X-100 and blocked in 1.5% normal goat serum. Sections were then incubated with the primary antibody rabbit anti-glia fibrillary acidic protein (Abcam, Cambridge, MA) at 1:1000 for astrocytes or rabbit anti-IBα (Wako, Osaka, Japan) at 1:250 for microglia overnight at 4°C. The secondary antibody Alexa 488–labeled goat anti-rabbit (Jackson Immunoresearch, West Grove, PA) diluted to 1:200 was applied for 1 hour. Then Aβ was stained using 0.05% thioflavin-S. In addition, brain and kidney sections from control and gelsolin-treated Tg2576 mice were stained for gelsolin using the primary antibodies mouse anti-bovine gelsolin (Sigma-Aldrich) at 1:500. Kidney sections from control mice were also used for immunostaining of gelsolin using a mouse anti-mouse gelsolin (Biorbyt Riverside, Cambridge, UK) diluted to 1:500. This was used in conjunction with an M.O.M. detection kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions.
ELISA
The concentration of Aβ40 in plasma from untreated, LY-411575–treated, and gelsolin/LY-411575–treated mice was quantified using a colorimetric human/rat Aβ40 ELISA kit (Wako) according to the manufacturer’s instructions. Blood samples were taken via cardiac puncture on Day 21 and were collected in Eppendorf tubes treated with 10 μL of ethylenediaminetetraacetic acid (10 mg/mL). Samples were centrifuged at 3,500 rpm for 7 minutes and stored at −80°C until processed. Plasma samples, diluted 3-fold in sample diluent, were applied in triplicate to the precoated ELISA plate and incubated overnight at 4°C. The next day, the plate was thoroughly washed and incubated with horseradish peroxidase–labeled anti-Aβ40–specific antibody BA27 for 1 hour at room temperature. Then, 3,3'-diaminobenzidine chromogen was used for detection of the horseradish peroxidase–bound antibodies. Synthetic Aβ40 standard solution was used for construction of calibration curves in the range of 1 to 100 pM and, hence, for determining Aβ concentrations in the plasma samples.

Statistical Analysis
We used mixed-effects models of CAA burden as a function of time to analyze the rate of progression of vascular amyloid deposition as previously described (16). The model included random-effects terms to account for both interanimal variation and vessel-to-vessel variation within each animal. To assess the effect of treatment, we fit a broken-line regression model that allowed for fixed treatment-specific slopes (i.e., progression rates) that could be different after Day 7 versus before Day 7, as well as during a 21-day period. Before conducting this study, we estimated the power that we would obtain from a design that would use 3 to 4 mice per group and 4 to 5 vessels per mouse. To do this, we simulated the experiment based on estimates of residual error, intervessel variability, and intermouse variability from our previous studies. We then fit the mixed-effects longitudinal models to the simulated data and assessed significance at the 0.05 level of the difference between control and treated progression rates. We found that we had a statistically high power with this design to detect differences in progression rates of the expected magnitude with animal cohort sizes of 3 to 4.

RESULTS
Gelsolin Slows CAA Progression in Tg2576 Mice
To assess the effect of gelsolin on the rate of deposition or clearance of CAA, we administered gelsolin to APP mice peripherally at a dose previously reported to reduce amyloid in the brain (8). As previously demonstrated, vessel segments from untreated mice showed a linear increase in CAA deposition throughout the 21-day observation period (Fig. 1). In gelsolin-treated animals, the rate of CAA progression in the first week (before treatment) was similar to that in untreated animals (0.38% vs 0.31% per day; not significant). In the treated period (between Day 7 and Day 21), however, the rate of CAA progression was markedly diminished (gelsolin, 0.11% per day vs untreated, 0.58% per day; p = 0.04 by linear mixed-effects model) (Fig. 1; Table 1, and it was not statistically different from the rate at Day 0. Thus, peripheral gelsolin treatment prevented CAA progression in the CNS, although it did not lead to reversal of existing amyloid deposits in this time frame.

Additional experiments were performed to confirm that the administered gelsolin acted via a peripheral mechanism to allow clearance of Aβ out of the CNS. By immunohistochemistry, bovine gelsolin was detected in the kidney, but not the brain, of gelsolin-treated mice (Fig. 2), whereas endogenous mouse gelsolin was not detected in either the kidney or the brain of untreated mice (Fig. 2). To verify that gelsolin treatment did not induce a neuroinflammatory response, brain sections from both gelsolin-treated and untreated mice were immunostained with markers for astrocytes (gliarial fibrillary acidic protein) and microglia (Iba-1). No detectable increases in astrocytes or microglia were observed in gelsolin-treated mice compared with those in untreated mice (Fig. 3). These results indicate that gelsolin is excluded from the CNS after peripheral administration (as previously reported), and that the effect on CAA progression is not likely to be secondary to a neuroinflammatory response.

Inhibition of γ-Secretase Reduces CAA Progression in Tg2576 Mice
The previous experiments addressed the role of clearance of amyloid on CAA progression. To address the role of prevention of formation of new amyloid on CAA progression, we used an experimental γ-secretase inhibitor (LY-411575) that has been shown to be effective in reducing Aβ levels in the brain, plasma, and CSF of Tg2576 mice (12). Treatment in these experiments started at Day 0. Vessel segments from LY-411575–treated mice showed a markedly reduced rate of progression relative to those from vehicle-treated animals (−0.17% vs 0.52%; p < 0.0001; Fig. 4; Table 2). Although there was a trend for modest regression of CAA throughout this treatment period, the rate was not statistically different from that at Day 0 (p = 0.07) (Table 2).

To confirm that the LY-411575 dose used was effectively inhibiting Aβ production, plasma Aβ was measured using ELISA (expressed as picomoles per liter; mean ± SEM). Plasma Aβ levels were reduced by approximately 70% in LY-411575–treated mice versus control mice (LY-411575, 223 ± 119 pM vs control, 807 ± 127 pM; p = 0.037).

Combined Gelsolin/LY-411575 Treatment Does Not Promote Further Clearance of CAA in Tg2576 Mice
Because gelsolin and LY-411575 both alter brain Aβ levels via different mechanisms (clearance via peripheral binding and inhibition of production, respectively) and both reduce the rate of progression of CAA, it is conceivable that combined treatment would not only reduce progression but also lead to clearance of CAA. To address this hypothesis, Tg2576 mice received both gelsolin and LY-411575 during a 3-week period, administered intraperitoneally daily. Vessel segments from treated mice showed markedly reduced progression of CAA deposition during the 21-day treatment period (Fig. 4A) versus progression rates in control mice (gelsolin/LY-411575, −0.004% per day vs control, 0.52% per day; p < 0.0003; Table 2). However, combined gelsolin/LY-411575 treatment...
did not induce regression of CAA because progression rates (−0.004%) were not significantly different from Day 0 progression rates (p = 0.97). Plasma Aβ levels from mice receiving both gelsolin and LY-411575 were also significantly reduced compared with those from control mice (gelsolin/LY-411575, 117 ± 48 pM vs control, 807 ± 127 pM; p = 0.044). These results indicate no additive or synergistic effect of a combined treatment of gelsolin and LY-411575 on the progression of CAA.

**DISCUSSION**

Immune-based AD therapies have shown some potential in reducing Aβ deposition in the form of senile plaques (22, 23); however, these treatments have had a limited effect on reducing the accumulation of vascular Aβ (24) and caused adverse complications, including meningoencephalitis, microhemorrhages, and vasogenic edema (4–6). We assessed the ability of 2 non–immune-based agents to reduce the deposition of vascular Aβ by altering the concentration of soluble Aβ levels in the brain. One of the advantages of the in vivo multiphoton microscopy technique is that large groups of animals (as for a parallel study) are not necessary. With this approach, we have shown that the Aβ-binding agent gelsolin and the γ-secretase inhibitor LY-411575 were able to stop the progression of CAA in Tg2576 transgenic mice, although there was no evidence of Aβ clearance of the CAA burden over time. This limited effect may account for the lack of synergistic effect when both drugs (gelsolin and LY-411575) were combined. It is important to note that neither treatment is specific for Aβ, allowing the possibility that alternative mechanisms may impact CAA progression. Likewise, the mouse models may not fully recapitulate the dynamics of human CAA. Together, however, our results provide support for candidate approaches to preventing the progression of CAA and thereby potentially reducing the risks of intracerebral hemorrhage.

The 2 treatments we used target the Aβ peptide via inhibition of production and peripheral sequestration. The low-molecular-weight γ-secretase inhibitor LY-411575 is able to

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<th>Treatment</th>
<th>Days 0–7</th>
<th>Days 7–21</th>
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<tr>
<td>Gelsolin (from Day 7)</td>
<td>0.31 ± 0.29</td>
<td>0.11 ± 0.18*</td>
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Progression rates are expressed as the percentage of affected vessel per day ± SE.
*p = 0.04 versus untreated on Day 7 to Day 21.
FIGURE 2. Immunohistochemical staining for gelsolin in mouse kidney and brain sections from untreated and gelsolin-treated Tg2576 mice. There is positive staining for gelsolin (green) in the kidney section from the gelsolin-treated mouse.

Anti-mouse Gelsolin (control animal -kidney)

Anti-bovine gelsolin

Kidney

Brain

Control

Treated
cross the blood-brain barrier (11, 25) and prevent the C-terminal cleavage of the transmembrane polypeptide APP. It has been shown to prevent the generation of Aβ40 and Aβ42 peptide fragments in the CNS in animal models (26, 27) and in AD patients (28). It is important to recognize that current γ-secretase inhibitors also impede the cleavage of other substrates (29), among which Notch is of special relevance. However, side effects related to Notch inhibition might be controlled by adjusting dosage and length of the treatment (12, 30). In our hands, LY-411575-treated mice did not show the “sickly” phenotype previously described (12, 30). Ultimately, more specific inhibitors or γ-secretase modulators need to be developed. Preventing APP cleavage is a promising option for reducing the level of Aβ that is produced in the brain, leading to prevention of Aβ aggregation. Several studies using transgenic mouse models have shown significant reductions in brain, plasma, or CSF Aβ concentrations after treatment with γ-secretase inhibitors (13, 31). Our present observations using the inhibitor LY-411575 are in agreement with these findings, whereby plasma Aβ levels were significantly reduced, and extend them by demonstrating that progression of vascular Aβ deposition was impeded. There was no evidence that treatment with LY-411575 cleared existing vascular Aβ, however, and although we did not specifically quantify effects on senile plaques, we did not observe an obvious effect of LY-411575 on parenchymal Aβ. These observations are analogous to a previous report in which LY-411575 had no effect on the size or clearance of preexisting plaques of APP/PS1 mice (13). We confirmed that γ-secretase inhibition prevents the generation of newly formed Aβ aggregates, thereby stopping the progression of vascular Aβ deposition.

The actin-binding protein gelsolin is expressed both intracellularly and as a secretory protein found in CSF and plasma (32). It has previously been reported that extracellular gelsolin can act as a peripheral sink for soluble Aβ in plasma and CSF, thereby preventing its aggregation and causing

**FIGURE 3.** Immunohistochemical staining for astrocytes (anti–glial fibrillary acidic protein [GFAP] antibody, red), microglia (Iba1 antibody, red), and plaques (thioflavin S, green) in brain sections of untreated and gelsolin-treated Tg2576 mice. No difference in the level of astrocyte or microglial immunostaining is detected between the brains of untreated and gelsolin-treated mice.
defibrillation of preformed Aβ fibers (33, 34). Maintaining Aβ in its soluble form has proven effective in reducing overall brain Aβ load, as gelsolin treatment significantly reduced the amount of aggregated Aβ in the brain of APP/PS1 mice (8, 35). Similarly, in the current study, gelsolin treatment prevented the accumulation of Aβ deposits in leptomeningeal vessels of Tg2576 mice. Immunohistochemical analysis confirmed that gelsolin was restricted to the periphery because expression could only be detected in peripheral organs and not in the brain. Likewise, there was no evidence of neuroinflammation after treatment. Our results with these 2 independent treatment approaches that effectively reduce soluble Aβ strongly suggest that lowering the concentration of free soluble Aβ is sufficient to prevent the progression of CAA.

Both LY-411575 and gelsolin seemed unable to clear preexisting vascular Aβ deposits even when administered as a combined therapy. The only established method for removal of existing CAA is via direct binding of anti-Aβ antibodies (15, 36). Antibody-based therapies have been shown to have differential effects on the clearance of vascular and plaque Aβ. Short-lived exposure to antibody can clear plaque Aβ without reappearance (37, 38); conversely, vascular Aβ requires continuous exposure to prevent the deposits from returning (15). These observations suggest that vascular Aβ deposits differ in their stability or mechanism of replenishment.

The inability of gelsolin to induce clearance of vascular Aβ alone or in combination with LY-411575 may be caused by its ineffectiveness in binding the fibrillar form of Aβ. Initial reports claiming that gelsolin could defibrillize preformed Aβ fibrils relied on a synthetic form of Aβ (34) and led to reduced levels of amyloid in the brain (8, 39). It is conceivable that gelsolin could affect the prevention of new Aβ plaques but remain ineffective in clearing existing Aβ deposits. Gelsolin also has a lower affinity for Aβ compared

![Figure 4](http://jnen.oxfordjournals.org/) Serial in vivo imaging and quantitative analysis of CAA progression in LY-411575 and gelsolin/LY-411575–treated Tg2576 mice. (A) Representative in vivo images of individual CAA-laden leptomeningeal vessels in LY-411575 and gelsolin/LY-411575–treated Tg2576 mice on Day 0 and Day 21 of treatment. Angiograms were obtained to identify vessel region (blue); Aβ deposits were identified using systemic administration of methoxy-X04 (pink). Scale bar = 100 μm. (B) Quantitative measurements of CAA burden calculated as the percentage of vessel area affected by CAA in LY-411575–treated (n = 12 vessel segments from 3 mice) and gelsolin/LY-411575–treated (n = 10 vessel segments from 3 mice) Tg2576 mice on Day 0, Day 7, Day 14, and Day 21. Both treatments were initiated on Day 0.

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<tr>
<th>Treatment</th>
<th>Days 0–21</th>
<th>p*</th>
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<tr>
<td>Untreated</td>
<td>0.52 ± 0.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LY-411575†</td>
<td>−0.17 ± 0.09</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>Gelsolin and LY-411575†</td>
<td>−0.004 ± 0.10</td>
<td>&lt;0.0003</td>
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Progression rates are expressed as the percentage of affected vessel per day ± SEM. *Values of p are for treated versus untreated mice.

†Treatment from Day 0.
with Aβ antibodies, which may explain its reduced ability to clear fibrillar Aβ (40).

There are currently no treatments to prevent the major clinical manifestations of advanced CAA, including hemorhagic stroke, microinfarcts, and white matter lesions (3). Immune-based therapies have been the most effective approach to binding and clearing fibrillar Aβ deposits from the vascular wall despite potential adverse side effects (5, 6, 15, 36, 41). Agents that specifically reduce the production of Aβ or prevent aggregation in the brain (peripheral sequestration) have not yet reached the same extent of clinical evaluation (7, 8, 13). These therapies may be less prone to the adverse complications observed with fibrillar-binding antibodies because of the absence of an inflammatory response. Therefore, they represent rational therapeutic approaches for preventing the progression of CAA and reducing the risk of CAA-related complications.

REFERENCES
12. Hyde LA, McHugh NA, Chen J, et al. Studies to investigate the in vivo therapeutic window of the gamma-secretase inhibitor N2-[2S]-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1-[7S]-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl-L-alaninamide (LY-411575) in the CRND8 mouse. J Pharmacol Exp Ther 2006;319:1133–43
27. Best JD, Jay MT, Otu F, et al. Quantitative measurement of changes in amyloid-beta(40) in the rat brain and cerebrospinal fluid following treatment with the gamma-secretase inhibitor LY-411575 [N2-[2S]-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1-[7S]-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl-L-alaninamide]. J Pharmacol Exp Ther 2005;313:902–8
29. Schor NF. What the halted phase III gamma-secretase inhibitor trial may (or may not) be telling us. Ann Neurol 2011;69:237–39


