Generation of $A\beta_{38}$ and $A\beta_{42}$ Is Independently and Differentially Affected by Familial Alzheimer Disease-associated Presenilin Mutations and $\gamma$-Secretase Modulation*

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Alzheimer disease amyloid $\beta$-peptide ($A\beta$) is generated via proteolytic processing of the $\beta$-amyloid precursor protein by $\beta$- and $\gamma$-secretase. $\gamma$-Secretase can be blocked by selective inhibitors but can also be modulated by a subset of non-steroidal anti-inflammatory drugs, including sulindac sulfide. These drugs selectively reduce the generation of the aggregation-prone 42-amino acid $A\beta_{42}$ and concomitantly increase the levels of the rather benign $A\beta_{38}$. Here we show that $A\beta_{42}$ and $A\beta_{38}$ generation occur independently from each other. The amount of $A\beta_{42}$ produced by cells expressing 10 different familial Alzheimer disease (FAD)-associated mutations in presenilin (PS) 1, the catalytic subunit of $\gamma$-secretase, appeared to correlate with the respective age of onset in patients. However, $A\beta_{38}$ levels did not show a negative correlation with the age of onset. Modulation of $\gamma$-secretase activity by sulindac sulfide reduced $A\beta_{42}$ and increased $A\beta_{38}$ in the case of wild type PS1 and two FAD-associated PS1 mutations (M146L and A285V). The remaining eight PS1 FAD mutants showed either no reduction of $A\beta_{42}$ or only rather subtle effects. Strikingly, even the mutations that showed no effect on $A\beta_{42}$ levels allowed a robust increase of $A\beta_{38}$ upon treatment with sulindac sulfide. Similar observations were made for fenofibrate, a compound known to increase $A\beta_{42}$ and to decrease $A\beta_{38}$ for mutants that predominantly produce $A\beta_{42}$, the ability of fenofibrate to further increase $A\beta_{42}$ levels became diminished, whereas $A\beta_{38}$ levels were altered to varying extents for all mutants analyzed. Thus, we conclude that $A\beta_{38}$ and $A\beta_{42}$ production do not depend on each other. Using an independent non-steroidal anti-inflammatory drug derivative, we obtained similar results for PS1 as well as for PS2. These in vitro results were confirmed by in vivo experiments in transgenic mice expressing the PS2 N141I FAD mutant. Our findings therefore have strong implications on the selection of transgenic mouse models used for screening of the $A\beta_{42}$-lowering capacity of $\gamma$-secretase modulators. Furthermore, human patients with certain PS mutations may not respond to $\gamma$-secretase modulators.

Alzheimer disease is the most abundant form of dementia, and increasing numbers of patients are to be expected in the near future. Amyloid $\beta$-peptide ($A\beta$) is a central player in the disease pathology. Originally it was purified as the building block of the disease-defining amyloid plaques. Now it is becoming clear that amyloid plaques are probably not the major neurotoxic entity in the disease rather this is an assembly of soluble oligomeric $A\beta$ species (1). These assemblies initiate the so-called amyloid cascade and finally induce abnormal phosphorylation of tau and subsequent formation of paired helical filaments (2). $A\beta$ is generated by proteolytic processing of the $\beta$-amyloid precursor protein (APP). Two proteases, $\beta$-secretase and $\gamma$-secretase, perform the cleavages on the N and C termini of the $A\beta$ domain, respectively (3). $\beta$-Secretase is a conventional aspartyl protease, whereas $\gamma$-secretase is a rather unusual aspartyl protease capable of intramembraneous cleavage by utilization of a novel active site (4) that is signified by a highly conserved GXGD motif that includes one of the two active site aspartyl residues (5). $\gamma$-Secretase is a complex composed of four subunits, presenilin 1 (PS1) or PS2, APP-1a or APP-1b, PEN-2, and nicastrin. PS harbors the catalytically active center of the protease. Numerous familial Alzheimer disease (FAD)-associated mutations occur within the PSs. They all increase the $A\beta_{42}/A\beta_{40}$ ratio. Since $A\beta_{42}$ aggregates much faster than $A\beta_{40}$ these mutations affect the kinetics of oligomer formation and aggregation (1).

5 The abbreviations used are: $A\beta$, amyloid $\beta$-peptide; APP, $\beta$-amyloid precursor protein; FAD, familial Alzheimer disease; NSAID, non-steroidal anti-inflammatory drug; PS, presenilin; WT, wild type; HEK, human embryonic kidney; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MS, mass spectrometry; GSM-1, $\gamma$-secretase modulator 1.
Pharmacological inhibition of the secretases is a major therapeutic task. Unfortunately, the development of β-secretase inhibitors seems to be rather complicated (6), and γ-secretase inhibitors caused major side effects in animal models and during clinical trials, due to the reduction of the biological function of γ-secretase in Notch signaling (3).

However, modulators of γ-secretase activity, namely a subset of the non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or sulindac sulfide, selectively reduce the production of the aggregation-prone Aβ42 while leaving Notch signaling intact (7–10). Concomitantly, they increase Aβ38 production (10). Therefore, there seems to be an equilibrium of Aβ42 and Aβ38 generation. Moreover, compounds such as fenofibrate, which are known to exhibit an opposite modulating activity by increasing Aβ38 production, reduce Aβ38 production (11), again suggesting an interdependence of Aβ42 and Aβ38 production. NSAIDs most likely modulate γ-secretase activity directly, because they are active in cell-free assays (7, 8, 12–14) and they are known to affect the conformation of PS probably by an allosteric mechanism (15). Moreover, their modulating activity in terms of Aβ42 reduction is affected by some FAD-associated PS1 mutations. The PS1 Δexon9 mutation decreases the sensitivity of the γ-secretase to sulindac sulfide, whereas the PS1 M146L mutation enhances its sensitivity (13).

We now investigated the equilibrium of Aβ38 and Aβ42 generation in cells expressing wild type (WT) PS or FAD-associated PS mutations as well as in transgenic mice expressing WT PS2 or a FAD-associated PS2 mutation. Surprisingly, we found that Aβ38 and Aβ42 generation do not depend on each other. Moreover, Aβ38 and Aβ42 respond differentially to γ-secretase modulation depending on the PS mutation expressed.

EXPERIMENTAL PROCEDURES

Antibodies—Monoclonal antibodies against the PS1 N terminus (PS1N) and against the PS2 large loop (BLHF5c), as well as the poly- and monoclonal antibodies to Aβ (3552, 2D8), were described previously (16). The C-terminal-specific anti-Aβ38 antibody was obtained from Meso Scale Discovery, and C-terminal-specific anti-Aβ40 (BAP24) and anti-Aβ42 (BAP15) antibodies were a kind gift from Dr. Manfred Brockhaus (Roche Applied Science).
cDNA Constructs—cDNA constructs encoding PS1 and PS2 FAD mutants were generated by PCR-mediated mutagenesis using oligonucleotide primers encoding the respective mutations and cloned into pcDNA3.1/zeo (+) (Invitrogen).

Cell Culture—Human embryonic kidney (HEK) 293 cells stably co-expressing Swedish mutant APP (HEK293/sw) together with the indicated PS variant were cultured as described before (17). HEK293/sw cells were stably transfected with the indicated PS cDNAs using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Pools of stably transfected PS cells were investigated to avoid clonal variations. Cells were plated at a density of 200,000 cells/well in poly-L-lysine-coated 24-well plates and incubated for 24 h. Thereafter, cells were incubated in 500 μl of fresh medium containing either 50 μM sulindac sulfide (Sigma), 100 μM fenobrate (Sigma), 5 μM GSM-1 (Roche Applied Science), or vehicle (Me2SO) for 16 h before analysis of conditioned medium by sandwich immunoassay. Treatments were performed in triplicate, and all media samples were measured in duplicate for Aβ38, Aβ40, and Aβ42.

PS Analysis—Cell lysates were obtained with STEN-lysis buffer (50 mM Tris, pH 7.6, 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, protease inhibitors (Sigma)) followed by ultracentrifugation at 100,000 × g for 30 min at 4 °C. Proteins were separated by 10% urea SDS-PAGE, and PS was analyzed by immunoblotting.

Mass Spectrometry Analysis—Immunoprecipitation-MS analysis of Aβ species was carried out as described previously (18). Briefly, Aβ species were immunoprecipitated from conditioned medium from each cell line with antibody 82E1 for 4 h at 4 °C. Immunoprecipitates were washed four times with immunoprecipitation-MS buffer (0.1% N-octylglucoside, 140 mM NaCl, 10 mM Tris, pH 8.0) and two times with distilled water. Immunoprecipitated peptides were eluted with 0.3% trifluoroacetic acid in 40% acetonitrile saturated with a-cyano-4-hydroxy cinnamic acid. The dissolved samples were dried on a stainless plate and subjected to MALDI-TOF MS analysis using Voyager DE STR (Applied Biosystems).

Quantification of Secreted Aβ—Secreted Aβ peptides in conditioned medium were quantified by a sandwich immunoassay using the Meso Scale Discovery Sector Imager 2400 reader. The corresponding concentrations of Aβ peptides were calculated using the Meso Scale Discovery Discovery Workbench software. Ratios of each Aβ species as a percentage of total Aβ (Aβ38, Aβ40, and Aβ42) were then calculated, and graphs were plotted using the GraphPad Prism software.

Compound Synthesis and Administration—The γ-secretase inhibitor MRK-560 (19–21) was synthesized starting from commercially available materials following the procedures depicted by Castro Pineiro et al. (22) and Churcher et al. (23). The γ-secretase modulator GSM-1 was synthesized starting from commercially available materials following the procedures depicted by Hannam et al. (24). Both compounds were dissolved in 5% ethanol, 10% solutol and administered by oral gavage at a dosing volume of 10 ml/kg.

In Vivo Experiments—Mice homozygous for huAPPsw (line 147.72H) (25) and mice homozygous for both huAPPsw and huPS2 mutant N141I (line B6.152H) (26) were used in these experiments. In these lines, the expression of human APPsw and human mutant PS2 is driven by the Thy-1 and prion promoters, respectively (25). Studies were conducted with mice aged 2–3 months, because at this age cortical Aβ is primarily in a soluble form (27). Mice were sacrificed 4 h after a single oral administration of the drugs or vehicle. Brains were collected and frozen on dry ice until analysis of soluble cerebral Aβ. All in vivo experiments were conducted in strict adherence to the Swiss federal regulations on animal protection and to the rules of the Association for Assessment and Accreditation of Laboratory Animal Care.

Extraction and Quantification of Cerebral Aβ—For determination of Aβ38, Aβ40, Aβ42, and total Aβ, brain hemispheres were homogenized in 9 volumes of 1.0% diethylamino/50 mM sodium chloride, incubated for 3 h on ice, and then centrifuged at 100,000 × g (1 h, 4 °C). Supernatants were aliquoted Plates were washed twice with phosphate-buffered saline-Tween before the addition of media samples and Aβ peptide standards (Bachem). Ruthenylated C-terminal-specific anti-Aβ38 (Meso Scale Discovery), anti-Aβ40 (BAP24), or anti-Aβ42 (BAP24) antibodies were diluted 1:1000 in blocking buffer and used as detection antibodies. Plates were incubated at room temperature for 2 h before washing twice with phosphate-buffered saline-Tween and twice with phosphate-buffered saline. For detection, Meso Scale Discovery Read buffer was added, and the light emission at 620 nm after electrochemical stimulation was measured using the Meso Scale Discovery Sector Imager 2400 reader. The corresponding concentrations of Aβ peptides were calculated using the Meso Scale Discovery Discovery Workbench software. Ratios of each Aβ species as a percentage of total Aβ (Aβ38, Aβ40, and Aβ42) were then calculated, and graphs were plotted using the GraphPad Prism software.

Independent Generation of Aβ38 and Aβ42 by γ-Secretase

FIGURE 1. PS1 FAD mutants differentially affect Aβ38 and Aβ42 generation. A, cell lysates of HEK293/sw cells expressing endogenous PS (Endo) and HEK293/sw cells stably overexpressing PS1 WT or the indicated PS1 FAD mutants were analyzed for PS1 derivatives (holoprotein and N-terminal fragment (NTF)) and PS2 C-terminal fragment (CTF) by immunoblotting. PS1 holoprotein is visible in all cells overexpressing PS1 mutants in addition to PS1 NTF (top panel). Note that PS1 Δexon9 does not undergo endoproteolysis. Stable expression of PS1 results in the displacement of PS2 CTFs (lower panel). B, sandwich immunoassay of Aβ38, Aβ40, and Aβ42 species that were isolated from conditioned medium of cells overexpressing PS1 WT and the indicated PS1 FAD mutations. Each species is plotted as a percentage of the total Aβ measured for each cell line. Data are arranged from left to right in order of Aβ42 levels, and this correlates well with the age of onset of the different mutations. Aβ42 levels are inversely correlated with Aβ42 levels, in contrast to Aβ42, which shows no correlation. For clarity, Aβ38 quantitation is enlarged in the lower panel. Data are plotted as the mean of three experiments with error bars indicating the S.E. C, qualitative MALDI-TOF MS of Aβ species produced by cells expressing PS1 variants. MALDI-TOF MS were performed on Aβ species immunoprecipitated from conditioned medium. Aβ38, Aβ40, and Aβ42 could be detected as distinct and most abundant Aβ species secreted by cells expressing all PS1 variants, and the spectra are consistent with the immunoassay data. The Aβ42 signal was low for PS1 WT but slightly increased for the mild mutations such as M146L, Δexon9, A285V, and L42F. For the severe mutations such as L166P, P117L, M235V, and Y256S, a peak corresponding to Aβ42 was more readily observed. Interestingly, for the mutations Δexon9, ΔIM, L166P, and Y256S, a peak corresponding to Aβ38 was also detected. Furthermore, the mutation M235V produced Aβ38 as a distinct species, although its amount compared with other species is less as investigated by gel electrophoresis (data not shown). However, Aβ38, Aβ40, and Aβ42 were the most abundant species secreted by the majority of PS1 variants.
and stored at −80 °C until assayed. Brain Aβ levels were determined using the Liquid Phase Electrochemiluminescent method as described in Narlawar et al. (28).

RESULTS

To study whether Aβ42 and Aβ38 production is coupled, we first investigated 10 selected PS1 FAD-linked mutations. Mutations were selected that cover a wide range of disease onsets (from ~50 years in the case of the PS1 M146L and A285V mutations to less than 30 years in the case of the PS1 P117L and Y256S mutations). cDNA constructs encoding PS1 mutations as well as WT PS1 were stably transfected into HEK293 cells stably expressing Swedish mutant APP to facilitate analysis of secreted Aβ species. Cell lysates were then investigated for the replacement of endogenous PS2, which served as an indicator for successful ectopic expression of PS1 (29). As shown in Fig. 1A, endogenous PS2 was fully replaced by PS1, demonstrating ectopic expression of all PS1 mutations. Due to overexpression, PS1 holoprotein accumulated for all mutants. In each case PS1 was endoproteolysed, with the exception of PS1 Δ exon9, which is known not to undergo proteolytic processing (29) (Fig. 1A).

We then determined the total levels of secreted Aβ38, Aβ40, and Aβ42. As expected, the PS1 mutations cover a wide spectrum of effects on Aβ42 production. This can range from a rather mild increase, as in the case of the PS1 M146L mutation, to a very severe increase as in the case of the PS1 L166P, G384A, M233V, P117L, and Y256S mutations (Fig. 1B). In line with our previous findings (17), the increase of Aβ42 production seems to occur at the expense of Aβ40, because these levels gradually decrease with increased Aβ42 levels. These findings are also largely consistent with an inverse correlation of Aβ42 levels with age of onset (30). In contrast, Aβ42 levels failed to show any negative correlation to Aβ43 levels (Fig. 1B, enlarged in lower panel), suggesting that Aβ42 is generated independently of Aβ43. Because alternative Aβ species with C termini other than amino acids 38, 40, or 42 could not be detected by the sandwich immunoassay, we also investigated Aβ species by qualitative mass spectrometry analysis. Although some additional species such as Aβ39 and Aβ43 were occasionally observed upon expression of certain mutations, Aβ38, Aβ40, and Aβ42 were the most abundant species for the majority of mutants (Fig. 1C).

To further investigate whether Aβ38 and Aβ42 production are directly linked to each other, we treated cells with the NSAID sulindac sulfide. Sulindac sulfide is known to modulate γ-secretase activity by shifting the cleavage from amino acid 42 to amino acid 38 (10). In line with previous findings, the PS1 M146L mutation showed slightly greater sensitivity to treat-
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FIGURE 4. Both PS1 and PS2 FAD-associated mutants respond differently to the γ-secretase modulator GSM-1 in respect to Aβ38 and Aβ42. A, structure of GSM-1. B, effect of 5 μM GSM-1 on Aβ42 (upper panel) and Aβ38 (lower panel) in cells expressing PS1 WT or the PS1 L166P mutation. PS1 WT responds to treatment with a significant increase in Aβ38 and a significant decrease in Aβ42. However, although L166P shows a stronger increase in Aβ38 upon treatment compared with PS1 WT, there is no reduction in Aβ42. C, effect of 5 μM GSM-1 on Aβ42 (upper panel) and Aβ38 (lower panel) in cells expressing PS2 WT or the PS2 N141I mutation (PS2 N141I). Both cell lines respond to treatment with similar increases in Aβ38, but there is no reduction in Aβ42 for PS2 N141I despite a robust decrease in Aβ42 for PS2 WT. Aβ42 levels are even slightly increased for PS2 N141I upon treatment with GSM-1. Data are plotted as the percentage change in Aβ species from vehicle-treated cells, which are normalized to 100% for each cell line. Error bars indicate the S.E., and statistical significance is calculated by one-way analysis of variance followed by Dunnett’s post-test. *, p < 0.05; ***, p < 0.001.

ment with sulindac sulfide, whereas the PS1 Δexon9 and PS1 L166P mutations failed to respond to the Aβ42-lowering activity (13, 31) (Fig. 2). Furthermore, almost all other mutations with the exception of PS1 A285V showed only very little lowering of Aβ42 levels (Fig. 2). This suggests that these mutations render γ-secretase resistant to the modulating activity of sulindac sulfide. However, when we investigated the levels of secreted Aβ38, we found that all PS1 mutations, including those that showed no reduction of Aβ42 upon sulindac sulfide treatment such as PS1 Δexon9, L424R, and L166P, increased Aβ38 generation. Thus, some mutant γ-secretase complexes exhibit a selective resistance to the Aβ42-lowering activity of sulindac sulfide but remain sensitive to the Aβ38-modulating activity. This effect remained robust up to 100 μM sulindac sulfide, the maximum concentration tolerated by the cells (data not shown).

Independent Aβ38 and Aβ42 production was further supported by treatment of cells with fenofibrate. Fenofibrate is a lipid-regulating drug that has been shown to exhibit opposite effects on γ-secretase modulation by increasing Aβ42 and decreasing Aβ38 as compared with NSAIDs like sulindac sulfide and indomethacin (11). Interestingly, all PS1 mutations were less sensitive to the Aβ42-increasing activity of fenofibrate compared with WT PS1 (Fig. 3). Moreover, the very aggressive mutations such as PS1 L166P, G384A, M233V, P117L, and Y256S showed only a minor increase of Aβ42 (Fig. 3). However, although some of the more aggressive mutations responded to a lesser degree with regard to the Aβ42-increasing activity, they showed similarly robust decreases of Aβ38 (Fig. 3). Other mutations, like PS1 Δexon9, Δ1M, L424R, and L166P, showed a somewhat diminished but still significant reduction of Aβ38 (Fig. 3). Thus, some PS1 mutations render the γ-secretase complex in a way that it becomes more or less resistant to Aβ42 modulation, but, strikingly, this occurs independently of the Aβ38-modulating activity.

The observed effects on γ-secretase modulation are not restricted to PS1 mutations and the use of sulindac sulfide. We tested a novel NSAID derivative with a lower IC50 for γ-secretase modulation (Fig. 4A, GSM-1). Treatment of cells expressing WT PS1 with as little as 5 μM of this compound caused a significant increase in Aβ38 and a significant decrease in Aβ42 (Fig. 4B). Consistent with sulindac sulfide treatment, cells expressing L166P showed no change in Aβ42 levels despite a highly robust and significant increase in Aβ38 (Fig. 4B). To determine whether this effect was specific for PS1, we investigated cells expressing WT PS2 and PS2 harboring the “Volga German” mutation (PS2 N141I). Treatment of cells expressing WT PS2 with GSM-1 caused a significant increase in Aβ38 and a significant decrease in Aβ42 (Fig. 4C); however, not much change in Aβ42 was observed for PS2 N141I despite a significant increase in Aβ38 (Fig. 4C). Interestingly, WT PS2 expressing cells showed a much stronger increase of Aβ38 compared with cells expressing WT PS1 (Fig. 4, B and C).

To prove that γ-secretase modulators differently affect Aβ42 and Aβ38 production in vivo, we compared the effect of the in vivo active modulator GSM-1 in transgenic mice expressing APP-Swe or APP-Swe/PS2 N141I (Fig. 5). The modulator GSM-1 significantly reduced brain Aβ42 levels (by 69% at 30 mg/kg) in the APP-Swe mice together with a corresponding increase of brain Aβ38 levels (>4-fold at 30 mg/kg) in a dose-dependent manner. No significant effect on brain Aβ40 could be observed (Fig. 5A). When tested in the double transgenic APP-Swe/PS2 N141I line, GSM-1 did not induce any significant reduction of brain Aβ42. However, it led to a significant dose-dependent increase of brain Aβ38. At the highest dose of 30 mg/kg, a significant reduction of brain Aβ40 of 27% could also be detected in the double transgenic line (Fig. 5B). Additionally, the effect of the γ-secretase inhibitor MRK-560 in these two transgenic lines is different for the mutant PS2;
**Independent Generation of Aβ_{38} and Aβ_{42} by γ-Secretase**

**A**

**APP-Swe**

![Graph](image)

**APP-Swe x PS2-N1411**

![Graph](image)

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**DISCUSSION**

Since the first description of the γ-secretase-modulating activity of NSAIDs, it was widely assumed that Aβ_{42} and Aβ_{38} generation are mutually dependent as decreased/increased Aβ_{42} production correlated with increased/decreased Aβ_{38} generation (10, 11). Our findings now demonstrate that Aβ_{38} generation is not coupled to Aβ_{42} production. This is shown by the lack of a negative correlation of Aβ_{38} levels with Aβ_{42} levels in the 10 different PS1 mutations investigated. Moreover, some FAD-associated PS mutations make the γ-secretase complex at least partially resistant to the Aβ_{38}-lowering activity of NSAIDs or the Aβ_{42}-increasing activity of fenofibrate, while they are still sensitive to the corresponding Aβ_{38}-modulating activity. There appears to be no correlation between the severity of the mutation and the responsiveness to Aβ_{38} modulation.

Currently, the cellular mechanisms that allow a selective modulation of these cleavages are not fully understood. However, studies using fluorescence resonance energy transfer methods suggest that NSAIDs allosterically affect the conformation of PS and, probably as a consequence, the PS1/APP interaction (15). Moreover, FAD-associated PS mutations also affect the structure of the active site by changing the spatial relation of the PS1 N- and C-terminal fragments, in a manner opposing the effect of Aβ42-lowering NSAIDs (15, 32). Finally, NSAIDs modulate γ-secretase activity in cell-free assays (7, 8, 12–14). All this suggests that NSAIDs directly or indirectly affect PS conformation and γ-secretase activity. However, other data imply that NSAIDs may affect intramembrane dimerization of APP and thereby influence γ-secretase processing at its variable sites (33). Our data support the idea that NSAIDs affect PS activity directly or indirectly. A conformational change induced by NSAIDs may affect substrate cleavage at selective sites, which is well known for FAD-associated PS mutations. If mutant PS adopts a certain pathological conformation, which in the case of the aggressive mutations allows production of very high levels of Aβ_{42}, this structure may not be changed any more by NSAIDs or fenofibrate. Under these conditions, further and efficient trimming of the substrate at position 38 of the Aβ domain may still be possible, thus still permitting the Aβ_{38}-modulating activity of NSAIDs. In addition, NSAIDs may affect substrate structure/position within the active site by changing its conformation.

Our data have strong implications for animal models selected for *in vivo* analysis of NSAIDs. So far, only transgenic mice with a FAD-associated APP mutation have been investigated, and in these cases NSAIDs show their expected activity (8, 11, 34). However, many laboratories use transgenic mice overexpressing APP in combination with more or less aggressive PS mutations to enhance and accelerate amyloid plaque formation. Our data from the PS2 N1411 mouse are in agreement with recent data from the PS1 L166P mouse (31). Such

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**FIGURE 5. Dose-dependent changes in brain Aβ following MRK-560 and GSM-1 administration.** A. APP-Swe mice show a dose-dependent increase in Aβ_{38} and decrease in Aβ_{42} upon treatment with GSM-1. Levels of Aβ_{38} and total Aβ levels are unchanged. B. APP-Swe x PS2 N1411 mice show no significant decrease in Aβ_{42} despite robust dose-dependent increases in Aβ_{38}. Total Aβ levels were unchanged, and Aβ_{40} levels were unchanged at 3 and 10 mg/kg GSM-1, although they were significantly reduced at 30 mg/kg GSM-1. Interestingly, the inhibitor MRK-560 is less efficacious in reducing Aβ_{42} levels in APP-Swe x PS2 N1411 mice. Vehicle, MRK-560 (3 mg/kg), or GSM-1 (3–30 mg/kg) were dosed orally, and concentrations of brain Aβ_{38}, Aβ_{40}, Aβ_{42}, and total Aβ were determined 4 h after treatment. All values are expressed as a percentage of vehicle-treated controls (n = 6 mice/group ± S.E., except for MRK-560, n = 3 ± S.E.). Statistical significance is calculated by one-way analysis of variance followed by Dunnett’s post-test. *, p < 0.05; **, p < 0.01. Note that brain Aβ_{38} levels were below limit of detection (<LOD) after treatment with MRK-560.

although brain Aβ_{40} and Aβ_{42} is reduced to the same extent (86 and 87%, respectively) in the single APP-Swe transgenic line, there is a pronounced difference in the APP-Swe/PS2 N1411 double transgenic line (95 and 44% for Aβ_{40} and Aβ_{42}, respectively). This result is consistent with a previous finding in mice transgenic for the PS1 L166P mutation (31), suggesting that reduced sensitivity to γ-secretase inhibitors in respect to Aβ_{42} production may be common among strong FAD-associated PS mutants. Finally, the modulator GSM-1 does not alter the total amount of brain Aβ, although total Aβ is reduced by the inhibitor MRK-560 (by 71% in the single transgenic line and by 64% in the double transgenic line).
models will lead to a false interpretation of the in vivo activity of NSAIDs and potentially other drugs, because the Aβ38-modulating activity of such drugs may be strongly compromised. Moreover, our findings also indicate that patients carrying aggressive PS1 or PS2 FAD mutations will not respond to NSAID treatment.

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