Sulindac Sulfide Is a Noncompetitive \(\gamma\)-Secretase Inhibitor That Preferentially Reduces \(\alpha\)B42 Generation*

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Nonsteroidal anti-inflammatory drugs (NSAIDs) have been known to reduce risk for Alzheimer's disease. In addition to the anti-inflammatory effects of NSAIDs to block cyclooxygenase, it has been shown recently that a subset of NSAIDs selectively inhibits the secretion of highly amyloidogenic \(\alpha\)B42 from cultured cells, although the molecular target(s) of NSAIDs in reducing the activity of \(\gamma\)-secretase for \(\alpha\)B42 generation (\(\gamma\)B42-secretase) still remain unknown. Here we show that sulindac sulfide (SSide) directly acts on \(\gamma\)-secretase and preferentially inhibits the \(\gamma\)B42-secretase activity derived from the 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate-solubilized membrane fractions of HeLa cells, in an in vitro \(\gamma\)-secretase assay using recombinant amyloid \(\beta\) precursor protein C100 as a substrate. SSide also inhibits activities for the generation of \(\alpha\)B40 as well as for Notch intracellular domain at higher concentrations. Notably, SSide displayed linear noncompetitive inhibition profiles for \(\gamma\)B42-secretase in vitro. Our data suggest that SSide is a direct inhibitor of \(\gamma\)-secretase that preferentially affects the \(\gamma\)B42-secretase activity.

Alzheimer's disease (AD)† is a dementing neurodegenerative disorder of the elderly characterized pathologically by neuronal loss in the cerebral cortex accompanied by massive deposition of amyloid \(\beta\) peptides (\(\alpha\)B) as senile plaques (1). \(\alpha\)B is produced by sequential proteolytic cleavages of the amyloid \(\beta\) precursor protein (\(\beta\)APP) by a set of membrane-bound proteases termed \(\beta\)- and \(\gamma\)-secretases. The C-terminal length of \(\alpha\)B generated by \(\gamma\)-secretase is heterogeneous; \(\alpha\)B42 is a relatively minor molecular species of the \(\alpha\)B secreted from cells, but it has a much higher propensity to aggregate and form amyloid compared with other \(\alpha\)B species. These findings provide strong support for the hypothesis that the deposition of \(\alpha\)B42 is closely related to the pathogenesis of AD, implicating \(\gamma\)-secretase as an important therapeutic target.

Mutations in \(PS1\) or \(PS2\) genes account for the majority of early onset familial AD, and these mutations cause an increase in the ratio or levels of production of \(\alpha\)B42 (1). It is known that PS is essential for the \(\gamma\)-secretase-mediated intramembranous cleavage not only for \(\beta\)APP but for other type I transmembrane proteins (e.g. Notch, ErbB4, E-cadherin, low density lipoprotein receptor-related protein, and CD44) (2). PS proteins undergo endoproteolysis to generate N- and C-terminal fragments and interact with other proteins (e.g. nicastrin, APH-1, and PEN-2) to form a high molecular weight (HMW) protein complex (3). The functional role of PS complex in \(\gamma\)-secretase activity still remains unknown. However, aspartyl protease transition state analogue inhibitors of \(\gamma\)-secretase, which harbors a hydroxyl ethylene isostere or a difluoro alcohol moiety, directly label PS fragments (4–6). In addition, a systematic analysis using a variety of PS mutants revealed that HMW complex formation of PS as well as conserved aspartyl residues within the transmembrane domain are essential for \(\gamma\)-secretase activity (7–11). Finally, in vitro \(\gamma\)-secretase activity is associated with PS HMW complex (12, 13). These data suggest that HMW PS complex corresponds to the \(\gamma\)-secretase, an atypical membrane-embedded aspartyl protease, and that PS proteins harbor the catalytic center for \(\gamma\)-secretase complex (3).

Epidemiological studies have shown that long term treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) prevents the development of AD (reviewed in Refs. 14 and 15). Recently, a prospective, population-based cohort study provided strong evidence that the long term use of NSAIDs significantly reduced the risk of AD (16). NSAIDs affect the inflammatory response by direct inhibition of cyclooxygenase (COX) enzymes. Moreover, recent studies indicate that NSAIDs are involved in transcriptional regulation by the modulation of IκB kinase \(\beta\) or peroxisome proliferator-activated receptors (PPAR) (reviewed in Ref. 17). It has been believed that NSAIDs might influence the AD pathology by inhibiting the inflammation response (e.g. activation of microglia and astroglia) in brains (14). In fact, administration of NSAIDs affects the inflamma-

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** This abbreviation used are: AD, Alzheimer's disease; \(\alpha\)B, amyloid \(\beta\) peptide; \(\beta\)APP, amyloid \(\beta\) precursor protein; CHAPS, 3-(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; COX, cyclooxygenase; DAPT, \(N\)-(\(\beta\)-difluoromethylacetyl)-\(l\)-alanyl\)-\(l\)-stereoisoglycine \(t\)-butyl ester; ELISA, enzyme-linked immunosorbent assay; NICD, Notch intracellular domain; NSAIDs, nonsteroidal anti-inflammatory drugs; mt, mutant; PS, presenilin; SSide, sulindac sulfide; SStone, sulindac sulfone; wt, wild-type; Bicine, \(N\)-\(N\)-bis(2-hydroxyethyl)-glycine; HMW, high molecular weight; AICD, APP intracellular domain; PPAR, peroxisome proliferator-activated receptors.
Inhibition of γ-Secretase Activity by Sulindac Sulfide

Fig. 1. Schematic representation of recombinant substrates used in this study. C100-FmH consists of an N-terminal Met, βAPP, (597–695) and the consecutive FLAG, -Myr, and His tags. Similarly, N102-FmH is composed of Notch-(1899–1798) with an N-terminal Met and C-terminal FLAG/Myc/His tag. De novo generated Aβ peptides and intracellular domains (black box and white boxes, respectively) were detected by two-site ELISAs (BAN50/BNT77/BA27/BC05) or using sensitive immunoblotting methods. The location of I716F mutation in mt C100-FmH is indicated by star.

**RESULTS**

**Characterization of the γ-Secretase Activity in Vitro**—We first characterized the biochemical and enzymatic properties of the γ-secretase activity as detected by our *in vitro* γ-secretase assay. For this purpose, we generated a recombinant protein substrate C100-FmH, based on the amino acid sequence of the C-terminal fragment of βAPP fused to FLAG-myc-His tag sequences at the C terminus (Fig. 1). *De novo* generation of Aβ peptides from recombinant C100-FmH incubated with membranes of HeLa cells as an enzyme source required the presence of 0.25% CHAPSO, whereas Triton X-100 or SDS abolished the γ-secretase activity (data not shown), which was consistent with the previous observations (12). Incubation of HeLa cell membranes with wild-type (wt) C100-FmH predominantly generated Aβ40, in addition to Aβ42 as a minor species (Fig. 2A). However, the relatively low levels of *de novo* generation of Aβ42 hampered detailed pharmacological and enzymatic analyses of Aβ42-generating activity from wt C100-FmH. Thus, we introduced the I716F mutation into the recombinant substrate, which had been described to cause a dramatic increase in Aβ42 generation in intact cell-based assays (27). *De novo* Aβ42 generation from I716F mutant (mt) C100-FmH was significantly increased, whereas the production of Aβ40 was almost totally abolished, suggesting that mt C100-FmH served as an optimal substrate for γ42-secretase (Fig. 2A). These proteolytic activities were recovered from the solubilized membrane fraction by 1% CHAPSO containing 1 mM NDSB-256. We next examined the effects of the two well characterized γ-secretase inhibitors, L-685,458 and DAPT, on Aβ generation in our *in vitro* assay (12, 23, 31). Both compounds inhibited Aβ-generating activities in a similar, concentration-dependent fashion (Fig. 2B). We then studied the Aβ-generating activities in membranes of various cell lines including embryonic fibroblasts derived from...
PS1/2 double knockout mice (9, 32). Consistent with the results of intact cell-based assays, de novo production of Aβ peptides was almost totally abolished in membrane fractions from PS1/2 double knockout cells, and these activities can be immunoprecipitated with antibodies against PS1 (Fig. 2C and data not shown). These data suggest that recombinant wt and mt C100-FmHs were processed by the endogenous, PS-dependent proteases. These data are consistent with the previous reports (12, 33, 34) on the in vitro γ-secretase assays using different types of recombinant C100 with or without C-terminal tags.

Intramembranous cleavage of γ-secretase substrates generates the intracellular domain fragments that are liberated from the membrane and mediate the signal pathways from plasma membrane to nucleus (2). Notably, a proteolytic generation of Notch intracellular domain (NICD) by PS-dependent γ-secretase is the most well known example, and potential side effects caused by the blockade of Notch pathway by γ-secretase inhibitors are emerging problems (35, 36). To analyze the γ-secretase activity to generate NICD in vitro, we generated a recombinant N102-FmH substrate composed of a 101-residue fragment of murine Notch1 beginning close to the S2 cleavage site and containing transmembrane domain fused to a FLAG-myc-His C-terminal tag (Fig. 1). After incubation of N102-FmH with the membrane fraction, we observed the appearance of an NICD-like polypeptide migrating slightly faster than N102-FmH, which was diminished by treatment with DAPT in a dose-dependent manner (Fig. 2F and data not shown). Thus, the recombinant N102-FmH polypeptide is also cleaved by
Inhibition of γ-Secretase Activity by Sulindac Sulfide

γ-secretase to generate C-terminally tagged NICD (NICD-FmH) in vitro, which was consistent with the recent report by Wolfe and colleagues (37, 38).

Effect of Sulindac Sulfide on γ-Secretase Activity in Vitro—It has been reported recently (21, 22) that a subset of NSAIDs lower Aβ42 without affecting Notch processing in cultured cells. To gain more insights into the effect of NSAIDs on APP and Notch processing, we have chosen three NSAIDs, i.e. sulindac sulfide (SSide), sulindac sulfone (SSone), and naproxen, to treat N2a NL/N cells stably coexpressing βAPPNL and NotchΔE (24). We confirmed a specific decrease in Aβ42 secretion by treatment with 10–30 μM SSide, whereas the secretion of Aβ40 as well as Notch processing was not affected (Fig. 3).

Treatment with 100 μM of SSide caused cell death presumably by inducing apoptosis, resulting in marked decrease in Aβ generation as well as in total protein expression (17). The IC50 value for Aβ42 secretion of SSide was 30.6 ± 2.8 μM. SSone and naproxen had no effect either on Aβ40 or Aβ42 secretion as well as on Notch cleavage up to 100 μM.

To examine whether SSide modulates γ-secretase activity by direct or indirect mechanisms (e.g. altering the trafficking of substrates or enzymes, affecting the secretion or degradation of Aβ42 peptides, or modifying the transcription of γ-secretase-related genes), we analyzed the in vitro γ-secretase activity in solubilized membrane fraction in the presence of NSAIDs. We observed an inhibition of γ42-secretase activity by SSide in a dose-dependent manner. The IC50 value of SSide for inhibiting γ42-secretase activity in vitro was 20.2 ± 2.6 μM (Fig. 4A). We found a decrease in slope by the increase of the concentration of SSide in the plot of rate against the enzyme concentration, suggesting that SSide is not an irreversible or pseudo-irreversible inhibitor (Fig. 4B). Moreover, when we dialyzed the solubilized γ-secretase fraction pretreated with SSide against CHAPSO buffer without SSide, γ-secretase activity was almost totally recovered (Fig. 4C). From these data, it was strongly suggested that the genuine molecular target of SSide is the γ-secretase complex and that SSide works as a reversible γ-secretase inhibitor.

In contrast to the results in cultured cells, the application of SSide at low concentrations (1–25 μM) caused a transient, but significant, increase in Aβ40 generation in vitro (Fig. 4A). Moreover, SSide diminished the de novo generation of Aβ40, in addition to that of Aβ42, at high concentrations (50–100 μM). Thus, SSide has an inhibition potency against γ40-secretase activity at high concentrations, whereas it elevates the γ40-secretase activity at sub-inhibitory doses. It was reported that the decrease in Aβ42 secretion by SSide was accompanied by a dose-dependent increase in Aβ38 secretion (21). To determine whether the decrease in Aβ42 (plus Aβ40) production caused by high concentrations of SSide affects that of Aβ38 in vitro, we analyzed the de novo generated Aβ species by high resolution immunoblotting (30). We observed a dose-dependent increase in Aβ38 generation in the low concentration range (1–25 μM) accompanied by a decrease in Aβ42 production, although the in vitro generation of Aβ peptides including Aβ38 was entirely inhibited by high concentrations of SSide (data not shown). These results suggest that SSide is a bona fide γ-secretase inhibitor directly affecting the membrane-embedded protease complex, exhibiting distinct inhibitory potencies against Aβ38-, Aβ40-, and Aβ42-generating activities of γ-secretase.

To characterize further the inhibitory mechanism of γ42-secretase activity by SSide, we performed the double-reciprocal plot analysis (Fig. 5A). We found that the Ks value remained at a constant level, but the Vmax value was decreased under increasing concentrations of SSide, suggesting that SSide displayed a noncompetitive inhibition for γ42-secretase activity. Because transition state analogue γ-secretase inhibitors (i.e. pepstatin or L-685,458) displayed linear noncompetitive inhibition profiles, a two-binding site model for intramembranous cleavage by γ-secretase has been proposed (34). In this model, γ-secretase complex is predicted to harbor a docking/anchoring site of substrates as well as a separate catalytic site. To determine whether SSide affects γ42-secretase activity by interacting with the catalytic site of γ42-secretase, we analyzed the inhibition profile of SSide by coinoculation with L-685,458, a transition state analogue inhibitor that is expected to occupy the active site of γ42-secretase. If SSide binds to the docking/anchoring site, coinoculation with SSide and L-685,458 would result in a synergistic inhibition of γ42-secretase and cause an increase in the inhibition slope. Unexpectedly, however, an addition of L-685,458 had no effect on the slope, raising the possibility that SSide may inhibit the γ42-secretase activity in a similar mechanism to that of a transition state analogue inhibitor, L-685,458 (Fig. 5B). Thus, SSide would compete for a
similar binding site (i.e. catalytic site) with L-685,458 in the γ-secretase complex or, alternatively, the binding of either of the inhibitors allosterically affects the interaction of the other.

We next analyzed the effect of SSide on intracellular domain generation from recombinant substrates of APP and Notch in vitro (Fig. 6). We observed that the inhibition kinetics of AICD-FmH generation from C100-FmH by SSide was approximately similar to that of the Aβ generation; the proteolytic activity to release AICD-FmH from wt C100-FmH was increased by treatment with 10–25 μM SSide, whereas it was completely inhibited at 100 μM. In contrast, AICD-FmH production from mt C100-FmH was inhibited by SSide in a dose-dependent fashion at 10–100 μM. We then analyzed the effect of SSide on N102-FmH generation by dialyzed membrane fraction preincubated with 100 μM SSide. Note that the dialysis of SSide-preincubated γ-secretase resulted in the almost total recovery of the enzymatic activity.
bition potency for Notch is much weaker than that for βAPP, especially for γ-secretase cleavage.

**DISCUSSION**

It has been shown that a subset of NSAIDs selectively lowers the secretion of Aβ42, although the molecular mechanism whereby NSAIDs affect the γ-secretase activity remained unclear (21, 22). In this study, we established an in vitro γ-secretase assay using recombinant wild type as well as mutant C100 as substrates and analyzed the effect of NSAIDs. We found that SSide, but not its metabolite SSone nor naproxen, directly inhibits the γ-secretase activity derived from membrane fractions of HeLa cells in a dose-dependent manner. Moreover, we showed that SSide is a bona fide γ-secretase inhibitor that has the highest inhibition potency against Aβ42-cleaving activity compared with those for Aβ38, Aβ40, or Notch, with noncompetitive inhibition kinetics.

It has been extensively documented that NSAIDs exhibit various molecular targets, of which the primary target is COX, that converts arachidonic acid to prostaglandins (17). In addition, SSide has been shown to inhibit IxK β kinase activity, activate PPARγ, inactivate PPARδ, inhibit Ras signaling, and reduce the proliferation and induce apoptosis of cancer cells (17). It has been shown that the Aβ42-lowering effect of NSAIDs is independent of COX-inhibiting activity in cultured cells (21). We confirmed the Aβ42-specific inhibition of Aβ secretion in culture cells by SSide, although the analysis of the inhibition profile at high concentrations was difficult because of the cell toxicity. To examine whether SSide directly inhibits γ-secretase, we employed an in vitro assay system using solubilized membranes as enzyme sources and recombinant C100 polypeptides as substrates, and we demonstrated that SSide has the capacity to inhibit the total γ-secretase activity, although cleavage at Aβ site was most effectively inhibited. In addition, in vitro Aβ generation took place in the absence of any NTPs, suggesting that kinase activities (e.g. IxK β kinase) are not involved in the regulation of γ-secretase by SSide; we also observed that addition of ATP does not change the level of de novo Aβ generation, suggesting that γ-secretase activity does not require energy. Finally, we showed that SSide displayed noncompetitive inhibition kinetics in vitro, which is a common characteristic of a number of γ-secretase inhibitors (34). From these data, we postulate that SSide works as a γ-secretase inhibitor that directly affects its activity by binding to the membrane-embedded protease complex.

To date, several γ-secretase inhibitors have been documented to inhibit secretion of Aβ40 and Aβ42 in two different patterns. The peptide aldehydes and peptidomimetic inhibitors containing a difluoroketone or alcohol group increase Aβ42 secretion at sub-inhibitory doses and diminish it at a high concentration, whereas they inhibit Aβ40 generation in an absolutely dose-dependent manner (39–41). However, the rank order of inhibitory potencies of several peptide aldehydes against Aβ40 and Aβ42 are at similar levels, suggesting that a single γ-secretase complex would generate Aβ40 and Aβ42 (40). In addition, the transition state analogue inhibitors of aspartyl proteases containing a hydroxyethylene isostere also inhibited the secretion of Aβ40 and Aβ42 to similar extents (12, 41). The compounds display similar inhibition kinetics between a cell-free system (i.e. incubation of the membrane fraction that harbors both enzyme and substrate) and an in vitro assay using recombinant substrates, suggesting that these compounds act directly on γ-secretase and that the differences in the inhibition kinetics might depend on their binding sites or target molecule(s) within the γ-secretase complex.

The molecular mechanism underlying the reciprocal regulation in Aβ40 and Aβ42 generation at low concentrations of peptide aldehyde inhibitors and SSide still remains unknown. One possible explanation is that a partial loss of γ-secretase function by the low concentrations of peptide aldehydes or difluoroketone peptide mimetics would shift the substrate supply to γ-secretase that is still active, thereby leading to overproduction of Aβ42. In sharp contrast, SSide exhibited entirely novel inhibition profiles to preferentially inhibit Aβ42 generation, which was accompanied by an increase in the production of Aβ38 as well as Aβ40 at sub-inhibitory concentration ranges in vitro. We speculate that SSide may act on a component that is distinct from those affected by peptide aldehydes or difluoroketone protease inhibitors. Alternatively, SSide and other inhibitors may exert opposite effects on a component that is involved in the determination of the position of a scissile bond to be cleaved in γ-secretase complex. Unexpectedly, a coincubation study with the transition state analogue inhibitor, L-685,458, showed a direct competition with SSide, raising the possibility that SSide might directly act on the catalytic site, although the structure of SSide
Inhibition of γ-Secretase Activity by Sulindac Sulfide

is not similar to any known substrates. An alternative possibility would be that SSIdc is closely related to the -secretase complex and allosterically regulates the catalytic site in a way to dissociate substrates and active site-specific inhibitors, showing an apparent direct competition. Such reciprocal regulation of different proteolytic activities by protease inhibitors or substrates has been observed in proteasome that harbors three distinct proteolytic activities (i.e. cysteinyl, trypanase- and peptidyl-glutamyl peptide-hydrolizing activities (42–44)).

Ritonavir, an inhibitor of human immunodeficiency virus-1 protease, competitively inhibits the chymotrypsin-like activity, whereas trypanase activity is enhanced (43). Extensive studies using active site-specific inhibitors suggested that proteasome effectors/substrates (e.g. ritonavir) that cause reciprocal regulation might act on noncatalytic sites, rather than through binding to an active site. Further studies using derivatives of SSIdc that contain affinity moieties (e.g. photoactive groups) are needed to obtain definitive proof that SSIdc acts directly on -secretase.

It has been documented that almost all -secretase inhibitors abolish the site-3 cleavage of Notch in cultured cells, with the exception of SSIdc and a nonpeptidic isocoumarin derivative, iritonavir (an inhibitor of human immunodeficiency virus-1 protease), whereas other peptidic inhibitors (e.g. DAPT) abolish Notch cleavage with similar potencies to those for Aβ generation. These results suggest that γ- and Notch secretases are pharmacologically distinct but related. Thus, it may be possible to avoid the envisaged side effects of -secretase inhibitors caused by inhibition of Notch signaling by developing derivatives of SSIdc. Further attempts to define the molecular mechanisms of inhibition on -secretase activity by SSIdc and to screen its derivatives specifically relevant to βAPP cleavage will facilitate not only the development of a novel therapeutic drug for AD but also our understanding of the unusual intramembranous proteolytic activity of -secretase that cleaves membrane-spanning proteins at multiple positions.

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REFERENCES

1. Selko, D. J. (2001) Physiol. Rev. 81, 741–766
2. Fortini, M. E. (2002) Nat. Rev. Mol. Cell Biol. 3, 673–684
3. Buxton, R. B., and Iwatsubo, T. (1998) Nat. Cell Biol. 12, 556–562
4. Li, Y. M., Lai, M. T., Xu, M., Huang, Q., DiMuzio-Mower, J., Sardana, M. K., Esler, W. P., Kimberly, W. T., Ostaszewski, B. L., Diehl, T. S., Moore, C. L., and Wolfe, M. S. (2002) J. Biol. Chem. 277, 3058–3058
5. Zaczek, R. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 5139–5144
6. Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G. M., Cooper, A. M., Coppola, D., Morgan, D., and Gordon, M. N. (2002) J. Neurosci. 22, 2246–2254
7. Tomita, T., Kataiyama, R., Takikawa, K., and Iwatsubo, T. (2002) FEBS Lett. 520, 117–121
8. Lim, G. P., Yang, F., Chu, T., Chen, P., Beech, W., Tran, T., Ubeda, O., Hoian Aske, K., Frautschy, S. A., and Cole, G. M. (2000) J. Neurosci. 20, 5709–5714
9. Li, Y. M., Ostaszewski, B. L., Ye, W., DiMuzio-Mower, J., and Wolfe, M. S. (2001) J. Neurosci. 21, 8387–8377
10. Steiner, H. (2002) Trends Cell Biol. 12, 405–417
11. Fortini, M. E. (2002) Nature Genet. 31, 3053–3058
12. Li, Y. M., Lai, M. T., Xu, M., Huang, Q., DiMuzio-Mower, J., Sardana, M. K., Esler, W. P., Kimberly, W. T., Ye, W., Ostaszewski, B. L., and Audia, J. E. (2001) J. Neurochem. 77, 217–218
13. Tomita, T., Kataiyama, R., Takikawa, K., and Iwatsubo, T. (2002) FEBS Lett. 520, 117–121.
14. Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G. M., Cooper, A. M., Coppola, D., Morgan, D., and Gordon, M. N. (2002) J. Neurosci. 22, 1515–1521
15. Tomed, J. P., Connor, K. E., DiCarlo, G., Wenk, G., and Kraus, J. L. (2000) J. Neurosci. 20, 8387–8377
16. Tomita, T., Kataiyama, R., Takikawa, K., and Iwatsubo, T. (2002) FEBS Lett. 520, 117–121.