Non-steroidal anti-inflammatory drugs have anti-amyloidogenic effects for Alzheimer's -amyloid fibrils in vitro

Non-steroidal anti-inflammatory drugs have anti-amyloidogenic effects for Alzheimer's ß-amyloid fibrils in vitro

Running title: Anti-amyloidogenic effects of NSAIDs

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Keywords: Alzheimer's disease; ß-amyloid fibrils; electron microscopy; non-steroidal anti-inflammatory drugs; thioflavin T.

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Abstract

The pathogenesis of Alzheimer's disease (AD) is characterized by cerebral deposits of amyloid ß-peptides (Aß) and neurofibrillary tangles which are surrounded by inflammatory cells.   Long-term uses of non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of developing AD and delay the onset of the disease.   In the present study, we used fluorescence spectroscopy with thioflavin T and electron microscopy to examine the effects of NSAIDs such as ibuprofen, aspirin, meclofenamic acid sodium salt, diclofenac sodium salt, ketoprofen, flurbiprofen, naproxen, sulindac sulfide and indomethacin on the formation, extension, and destabilization of ß-amyloid fibrils (fAß) at pH 7.5 at 37°C in vitro.   All examined NSAIDs dose-dependently inhibited formation of fAß from fresh Aß(1-40) and Aß(1-42), as well as their extension.   Moreover, these NSAIDs dose-dependently destabilized preformed fAßs.   The overall activity of the molecules examined was in the following order: ibuprofen ≈ sulindac sulfide ≥ meclofenamic acid sodium salt > aspirin ≈ ketoprofen ≥ flurbiprofen ≈ diclofenac sodium salt > naproxen ≈ indomethacin.   Although the mechanisms by which these NSAIDs inhibit fAß formation from Aß, and destabilize preformed fAß in vitro are still unclear, NSAIDs may be promising for the prevention and treatment of AD.

Keywords: Alzheimer's disease; ß-amyloid fibrils; electron microscopy; non-steroidal anti-inflammatory drugs; thioflavin T.

1. Introduction

In the pathogenesis of Alzheimer's disease (AD), the abundance of intraneural neurofibrillary tangles and the extracellular deposition of the amyloid ß-peptide (Aß) as amyloid plaques and vascular amyloid are seminal events (Selkoe, 2001).   The aggregation of Aß and ß-sheet formation are considered to be critical events that render these peptides neurotoxic (Pike et al., 1995).   The presence of chronic neuroinflammation also contributes to the protracted degenerative course of AD (McGeer and McGeer, 1995), and it is also common to other neurodegenerative disorders, such as Parkinson's disease and Creutzfelt-Jacob disease (Eikelenboom et al., 2002; Gao et al., 2003).   A chronic inflammatory response characterized by activated microglia, reactive astrocytes, complement factors, and increased inflammatory cytokine expression associated with Aß deposits has been described in the brain of AD patients (Rogers et al., 1996).   A number of epidemiological studies have demonstrated a reduced risk for AD in population with long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) (McGeer et al., 1996; in t'Veld et al., 1998; Akiyama et al., 2000; in t'Veld et al., 2001).

These epidemiological findings have been supported by experimental studies.   First, chronic ibuprofen (Ibu) treatment significantly diminished amyloid deposition (Lim et al., 2000), and improved behavioral impairment in the APPsw transgenic mouse (Tg2576) (Lim et al., 2001).   Second, a subset of NSAIDs such as Ibu, sulindac sulfide (SSide), indomethacin (Ind) (Weggen et al., 2001) and flurbiprofen (Flu) (Eriksen et al., 2003) selectively decreased the secretion of Aß(1-42) from cultured cells independently of cyclooxygenase (COX) activity and lowered the amount of soluble Aß(1-42) in the brains of Tg2576 mouse.   Third, SSide and enantiomers of Flu have been reported to target γ-secretase and preferentially reduce Aß(1-42) generation (Takahashi et al., 2003; Eriksen et al., 2003).

These data suggest that some of the NSAIDs may affect the pathogenetic process of AD directly.

However, except the preliminary study where Thomas et al. (2001) used the non-physiological short

peptide Aß(25-35), the effects of NSAIDs on the formation and destabilization of Alzheimer's

ß-amyloid fibrils (fAß) in vitro have not been studied in detail.

Using a nucleation-dependent polymerization model explaining the mechanism of fAß formation in

vitro (Jarrnet and Lansbury, 1993; Naiki and Gejyo, 1999), we previously found that

nordihydroguaiaretic acid (NDGA) and rifampicin (Rif) inhibit fAß formation from Aß and fAß

extention dose-dependently (Naiki et al., 1998).   Moreover, we reported that they also destabilize

fAß(1-40) and fAß(1-42) in a concentration-dependent manner within a few hours at pH 7.5 at 37°C,

based on fluorescence spectroscopic analysis with thioflavin T (ThT) and electron microscopic studies

(Ono et al., 2002b).

Here, we examined the effects of the major NSAIDs on the formation, extension, and

destabilization of fAß(1-40) and fAß(1-42) at pH 7.5 and 37°C in vitro, using fluorescence spectroscopy

with ThT and electron microscopy.

2. Methods

2.1.   Preparation of Aß and fAß solutions

Aß(1-40) (trifluoroacetate salt, lot number 540111 and 530108, Peptide Institute Inc., Osaka, Japan)

and Aß(1-42) (trifluoroacetate salt, lot number 540127 and 530914, Peptide Institute Inc., Osaka, Japan)

were dissolved by brief vortexing in 0.02% ammonia solution at a concentration of 500 μM (2.2

mg/mL) and 250 μM, respectively, in a 4°C room and stored at -80°C before assaying (fresh Aß(1-40)

and Aß(1-42) solutions).   fAß(1-40) and fAß(1-42) were formed from the fresh Aß(1-40) and

Aß(1-42) solutions, respectively, sonicated, and stored at 4°C as described elsewhere (Hasegawa et al.,

1999).

Fresh, non-aggregated fAß(1-40) and fAß(1-42) were obtained by extending sonicated fAß(1-40) or

fAß(1-42) with fresh Aß(1-40) or Aß(1-42) solutions, respectively, just before the destabilization

reaction (Ono et al., 2002a; 2002b) .   The reaction mixture was 600 μL and contained 10 μg/mL (2.3

μM) fAß(1-40) or fAß(1-42), 50 μM Aß(1-40) or Aß(1-42), 50 mM phosphate buffer, pH 7.5, and 100

mM NaCl.   After incubation at 37°C for 3-6 h under non-agitated conditions, the extension reaction

proceed to equilibrium as measured by the fluorescence of ThT.   In the following experiment, the

concentration of fAß(1-40) and fAß(1-42) in the final reaction mixture was regarded as 50 μM.

2.2. Fluorescence spectroscopy, electron microscopy, and polarized light microscopy.

A fluorescence spectroscopic study was performed as described by Naiki and Nakakuki (1996) on a

Hitachi F-2500 fluorescence spectrophotometer (Tokyo, Japan).   Optimum fluorescence measurement

of fAß(1-40) and fAß(1-42) were obtained at the excitation and emission wavelengths of 445 and 490

nm, respectively, with the reaction mixture containing 5 μM ThT (Wako Pure Chemical Industries Ltd,

Osaka, Japan) and 50 mM of glycine-NaOH buffer, pH 8.5.   An electron microscopic study and

polarized light microscopic study of the reaction mixtures were performed as described elsewhere

(Hasegawa et al., 1999).

2.3. Polymerization assay

Polymerization of Aß with or without fAß added as seeds was assayed as described elsewhere (Naiki et al., 1998).   The reaction mixture contained 50 µM Aß(1-40), or 25 or 50 µM Aß(1-42), 0 or 10 µg/mL fAß(1-40) or fAß(1-42), 0, 0.01, 0.1, 1, 10, or 50 µM NSAIDs (Ibu, aspirin (Asp), meclofenamic acid sodium salt (Mec), diclofenac sodium salt (Dic), ketoprofen (Ket), Flu, naproxen (Nap), SSide, Ind), NDGA, Rif, or nicotine (Sigma Chemical Co., St. Louis, MO, USA), 1% dimethyl sulfoxide (DMSO) (Nacakai Tesque, Inc., Kyoto, Japan), 50 mM phosphate buffer, pH 7.5, and 100 mM NaCl.   NSAIDs dissolved in DMSO at concentrations of 1, 10, 100 µM, 1 mM and 5 mM, were added to the reaction mixture to make the final concentration 0.01, 0.1, 1, 10 µM and 50 µM, respectively.

Aliquots (30 µL) of the mixture were put into oil-free PCR tubes (size: 0.5 mL, code number: 9046; Takara Shuzo Co. Ltd, Otsu, Japan).   The reaction tubes were then put into a DNA thermal cycler (PJ480; Perkin Elmer Cetus, Emeryville, CA, USA).   The plate temperature was elevated at maximal speed, starting at 4˚C, to 37˚C.   Incubation times ranged between 0 and 8 days (as indicated in each figure), and the reaction was stopped by placing the tubes on ice.   The reaction tubes were not agitated during the reaction.   From each reaction tube, triplicate 5 µL aliquots were removed, then subjected to fluorescence spectroscopy and the mean of each triplicate determined.   In the ThT solution, the concentration of NSAIDs examined in this study was diluted up to 1/200 of that in the reaction mixture.

We confirmed that these compounds did not quench ThT fluorescence at the diluted concentration (data not shown).

2.4. Destabilization assay

Destabilization of fAß was assayed as described elsewhere (Ono et al., 2002b). Briefly, the reaction mixture contained 25 μM fresh fAß(1-40) or fAß(1-42), 0, 0.01, 0.1, 1, 10 or 50 μM NSAIDs, NDGA, Rif, or nicotine, 1% DMSO, 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 1% (wt/vol) polyvinyl alcohol (Wako Pure Chemical Industries Ltd) to avoid the aggregation of fAß and the adsorption of fAß onto the inner wall of the tube during the reaction.

After being mixed by pipetting, triplicate 5 μL aliquots were subjected to fluorescence spectroscopy and 30 μL aliquots were put into oil-free PCR tubes (Takara Shuzo Co. Ltd, Otsu, Japan). The reaction tubes were then transferred into a DNA thermal cycler (Perkin Elmer Cetus, Emeryville, California). Starting at 4°C, the plate temperature was elevated at maximal speed to 37°C. Incubation times ranged from 0 to 24 h (as indicated in each figure), and the reaction was stopped by placing the tubes on ice. The reaction tubes were not agitated during the reaction. From each reaction tube, 5 μL aliquots in triplicate were subjected to fluorescence spectroscopy and the mean of the three measurements was determined. ThT fluorescence did not change significantly when fAß were incubated with 0-50 μM of NSAIDs at either 4°C or 37°C for 1 min, then subjected to ThT assay, indicating that these compounds did not compete with ThT for fAß (data not shown).

2.5. Other analytical procedures

Protein concentrations of Aßs and fAßs in the reaction mixtures were determined by the method of

Bradford (1976) with a protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA).   The Aß(1-40)

solution quantified by amino acid analysis was used as the standard.   The linear least squares fit and

one-way analysis of variance, post-hoc test by Scheffe were used for statistical analysis.

3. Results

3.1. Effect of NSAIDs on the kinetics of fAß polymerization

As shown in Fig. 1A-D, the fluorescence of ThT followed a characteristic sigmoidal curve when

fresh Aß(1-40) or Aß(1-42) was incubated at 37°C.   This curve is consistent with a

nucleation-dependent polymerization model (Jarrett and Lansbury, 1993; Naiki and Gejyo, 1999).

Fresh fAß(1-40) assumed a non-branched, helical filament structure of approximately 10 nm width and

exhibited a helical periodicity of approximately 220 nm.   In the fresh fAß(1-42) solution, two types of

filaments of approximately 8 and 12 nm width were observed.   Although the Aß batches used here

were different from those in our previous experiments, these morphologies of fAßs were similar to

those reported previously (Naiki and Nakakuki, 1996; Naiki et al., 1998).   Both fAß(1-40) and

fAß(1-42) stained with Congo red showed typical orange-green birefringence under polarized light.

The final equilibrium level of Aß(1-40) or Aß(1-42) was lowered dose-dependently by incubation with

10 and 50 μM Ibu, Ket or Nap (Fig. 1A-D).   Other NSAIDs (Asp, Mec, Dic, Flu, SSide and Ind) also significantly inhibited the polymerization of Aß(1-40) and Aß(1-42) (Fig. 6A, B).

As shown in Fig. 2A-D, the fluorescence increased hyperbolically without a lag phase and proceeded to equilibrium much more rapidly by incubation of fresh Aß(1-40) or Aß(1-42) with fAß(1-40) or fAß(1-42), respectively, at 37˚C, than that without seeds (compare Fig. 1A, B, and 2A, B). This curve is consistent with a first-order kinetic model (Naiki and Nakakuki, 1996).   The final equilibrium level was dose-dependently decreased by incubation of Aß(1-40) or Aß(1-42) with fAß(1-40) or Aß(1-42), respectively, with 10 or 50 μM Ibu, Ket or Nap (Fig. 2A-D).   Other NSAIDs (Asp, Mec, Dic, Flu, SSide and Ind) also significantly inhibited the extension of fAß(1-40) and fAß(1-42) (data not shown).

As shown in Fig. 2E, at a constant fAß(1-40) concentration, a good linearity was observed between the Aß(1-40) concentration and the initial rate of fAß(1-40) extension both in the presence and absence of Ibu.   This linearity is again consistent with a first-order kinetic model and indicates that at each Aß(1-40) concentration, the net rate of fAß(1-40) extension is the sum of the rates of polymerization and depolymerization (Naiki and Nakakuki, 1996; Hasegawa et al., 2002).   In the presence of 10 μM Ibu, the slope of the straight line decreased to about 36%.

As shown in Fig. 3B, clear fibril extension was observed by electron microscopy when fresh Aß(1-40) was incubated with fAß(1-40) at 37˚C.   However, 50 μM Ibu completely inhibited the extension of sonicated fAß(1-40) (Fig. 3A, C).   Ibu inhibited the extension of fAß(1-42) and other NSAIDs (Asp, Mec, Dic, Ket, Flu, Nap, SSide and Ind) also inhibited the extension of fAß(1-40) and fAß(1-42) (data not shown).

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3.2. Effect of NSAIDs on the kinetics of fAß destabilization

As shown in Fig. 4A-D, the fluorescence of ThT was almost unchanged during the incubation of

fresh fAß(1-40) or fAß(1-42) at 37˚C without additional molecules.   On the other hand, the ThT

fluorescence decreased immediately after addition of NSAIDs to the reaction mixture.   As shown in

Fig. 4A-D, the ThT fluorescence of fAß(1-40) and fAß(1-42) decreased immediately after addition of

Ibu, Ket, Nap, in a concentration-dependent manner.   Other NSAIDs (Asp, Mec, Dic, Flu, SSide and

Ind) also significantly destabilized fAß(1-40) and fAß(1-42) (Fig. 6C, D).

After incubation of 25 μM fresh fAß(1-40) with 50 μM Ibu for 1 h, many short, sheared fibrils were

observed (Fig. 5B).   At 6 h, the number of fibrils was markedly decreased, and small amorphous

aggregates were occasionally observed (Fig. 5C).   Ibu destabilized fresh fAß(1-42) and other NSAIDs

(Asp, Mec, Dic, Ket, Flu, Nap, SSide and Ind) also destabilized preformed fAß(1-40) and fAß(1-42)

(data not shown).

As shown in Fig. 6, statistical analysis revealed a significant difference in the potency of 50 μM

NSAIDs for the anti-polymerization effect (A, B) and for the fibril destabilizing effect (C, D).   In both

the cases of fAß(1-40) and fAß(1-42), the potency of NSAIDs for the anti-polymerization effect was

similar to that for the fibril-destabilizing effect.   Additionally, similar anti-polymerization and

fibril-destabilizing effects were observed for both fAßs.   The overall activity of the molecules

examined was in the order of: Ibu ≈ SSide ≥ Mec > Asp ≈ Ket ≥ Flu ≈ Dic > Nap ≈ Ind.   We finally

compared the anti-amyloidogenic and fibril-destabilizing activity of SSide and Ibu with that of NDGA,

Rif, and nicotine (Fig. 7).   Although NDGA exhibited the potent anti-amyloidogenic and

fibril-destabilizing activity dose-dependently, the activity of nicotine was weak or negligible.   Rif,

SSide and Ibu exhibited similar dose-dependent activity.

After incubation with 50 μM Ibu for 4 h, fAß(1-40) and fAß(1-42) were stained with Congo red

much more weakly than fresh fAß(1-40) and fAß(1-42), but they all showed orange-green

birefringence under polarized light.   This means that a significant amount of intact fAß(1-40) and

fAß(1-42) still remains in the mixture after the reaction, as shown in Fig. 5C.   No proteins were

detected by the Bradford assay in the supernatant after centrifugation at 4°C for 2 h at 1.6×10^4 g.   This

implies that although Ibu could destabilize fAß(1-40) and fAß(1-42) to visible aggregates (Fig. 5C),

they could not depolymerize fAß(1-40) and fAß(1-42) to monomers or oligomers of Aß(1-40) and

Aß(1-42).   When fresh 50 μM Aß(1-40) or Aß(1-42) was incubated with 10 μg/mL of the pellet at

37°C, no increase in the fluorescence was observed for 6 h.   This implies that destabilized fAß(1-40)

and fAß(1-42) could not function as seeds.

4. Discussion

In this study, we demonstrated that various NSAIDs dose-dependently inhibited formation of fAß

from fresh Aß(1-40) and Aß(1-42), as well as their extension in vitro.   Moreover, these NSAIDs

dose-dependently destabilized preformed fAßs.

Thomas et al. (2001) suggested that the anti-aggregation effect of NSAIDs may be due to their

interaction with epitope 3-6 and/or 25-35 in Aß, which are considered crucial to Aß aggregation and

neurotoxicity. As shown in Fig. 2E, the extension of fAß(1-40) followed a first-order kinetic model

even in the presence of Ibu. The net rate of fAß(1-40) extension is the sum of the rates of

polymerization and depolymerization (Naiki and Nakakuki, 1996; Hasegawa et al., 2002). Thus, one

possible explanation for the finding in Fig. 2E may be that Ibu bound to the ends of extending

fAß(1-40) and increased the rate of depolymerization by destabilizing the conformation of Aß(1-40)

which has just been incorporated into the fibril ends. Alternatively, Ibu would bind to Aß(1-40) and

consequently decrease the rate of polymerization. Tomiyama et al. (1996) suggested that Rif binds to

Aß by hydrophobic interactions between its lipophilic ansa chain and the hydrophobic region of Aß,

thus blocking associations between Aß molecules leading to fAß formation. The anti-amyloidogenic

and fibril-destabilizing activity of tetracycline (Tc), small-molecule anionic sulphonates or sulphates,

melatonin, ß-sheet breaker peptides (iAß5) and nicotine may also be related to the propensity to bind to

the specific sites of Aß (Kisilevsky et al. 1995; Soto et al. 1996; Pappolla et al. 1998; Forloni et al. 2001;

Zeng et al. 2001). Thus, it may be reasonable to consider that NSAIDs could exhibit their

anti-amyloidogenic and fibril-destabilizing effects by directly binding to Aßs and/or fAßs.

Taking into consideration the findings we obtained previously by systematic in vitro studies, we

judged the overall activity of the anti-amyloidogenic molecules to be in the order of: tannic acid >>

NDGA = wine-related polyphenols (myricetin, morin, quercetin) >> Rif = Tc > poly(vinylsulfonic acid,

sodium salt) = 1,3-propanedisulfonic acid, disodium salt > iAß5 > nicotine (Ono et al., 2002a; 2002b;

2003; 2004a). In this study, we observed that the activity of SSide and Ibu is similar to that of Rif (Fig.

7).

Next, we considered the structure-activity relationships of NSAIDs and other anti-amyloidogenic

compounds.   The antioxidant compounds with hydroxyphenyl rings are suggested to bind specifically

to Aß and/or fAß, inhibit fAß formation and/or destabilize preformed fAß (Ono et al., 2002b; 2003;

2004a; 2004b; Li et al., 2004).   On the other hand, the NSAIDs examined in this study have no

hydroxyphenyl rings (Fig. 8).   In general, these NSAIDs have an aromatic-based hydrophobic

structure with some fused ring structures and methyl and/or carboxyl groups.   This structure might be

quite suitable for specifically binding to free Aß and subsequently inhibiting the polymerization of Aß

into fAß.   Alternatively, this structure might be suitable for a specific binding to fAß and subsequent

destabilization of the ß-sheet rich conformation of Aß molecules in fAß.   Further studies, such as

nuclear magnetic resonance experiments, are essential to reveal the exact structure-activity relationships

for NSAIDs and other organic compounds that exhibit anti-amyloidogenic and fibril-destabilizing

effects in vitro.

Clinically, Ibu has been used in the treatment of nerve-root compression pain.   Although their

penetration into the cerebrospinal fluid (CSF) is 0.9-1.5 % of the plasma level, the CSF level of Ibu

derivatives is the range 595-1241 ng h/ml in the patients taking oral doses of 800 mg Ibu (Bannwarth et

al., 1995).   This corresponds to 2884-6016 μM h of Ibu, which were higher than the concentration

used in the present study.   It is therefore conceivable that therapeutic doses of Ibu could provide a

concentration sufficient to achieve the anti-amyloidogenic effects.

Recent epidemiological studies have revealed that long-term and/or short-term use of some

NSAIDs may protect against AD (McGeer et al., 1996; in t'Veld et al., 1998; Akiyama et al., 2000; in

t'Veld et al., 2001).   Our study and several reports on the effects of NSAIDs may well explain this

correlation.   First, the major therapeutic effect of NSAIDs is believed to be due to their inhibition of

COX-1 and COX-2, leading to the suppression of prostaglandin synthesis and chronic

neuroinflammation contributing to the protracted degenerative course of AD (McGeer and McGeer,

1995; Akiyama et al., 2000).   Second, Weggen et al. (2001) recently suggested that a subset of

NSAIDs including Ibu, SSide, Ind and *R*-Flu directly affect amyloid pathology in the brains of

transgenic mouse by reducing the amount of soluble Aß(1-42) peptide levels independently of COX

activity.   They also suggested that NSAIDs subtly alter γ-secreatase activity without significantly

perturbing other amyloid precursor protein (APP) pathways or Notch cleavage.   Some NSAIDs were

reported to lower Aß(1-42) levels selectively both in cultured cell and broken cell assays, suggesting

that these compounds interact directly with the γ-secretase complex itself that generates Aß from human

APP (Sagi et al., 2003; Eriksen et al., 2003).   In addition, a subset of NSAIDs can reduce Aß(1-42)

through inhibition of Rho activity, which may regulate APP processing in a cell culture (Zhou et al.,

2003).   Finally, as shown in this paper, NSAIDs dose-dependently inhibit fAß formation from fresh

Aß and their extension, as well as destabilize preformed fAß in vitro.   Thus, it may be reasonable to

speculate that NSAIDs could prevent the development of AD, not only through suppressing chronic

neuroinflammation and reducing the amount of soluble Aß(1-42) peptide levels, but also through

directly inhibiting the deposition of fAß in the brain.   In relation to the latter scenario, it may be

essential to evaluate the effect of NSAIDs on the Aß oligomer formation in a future study, because the

Aß oligomer has been reported to be the most neurotoxic form of all the Aß aggregates (Gong et al.,

2003).   Yang et al. (2005) recently reported that curcumin prevented Aß42 oligomer formation and

toxicity between 0.1 and 1.0 μM, which is consistent with our EC_{50} values of curcumin for the

anti-amyloidogenic and fibril-destabilizing activity (Ono et al., 2004b).   Although the exact

mechanisms of anti-amyloidogenic and fibril-destabilizing activity of NSAIDs are unclear, the careful and safe use of them could contribute the prevention and therapy of AD and other kinds of human amyloidosis.

Acknowledgements

References

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., Cooper, N.R., Eikelenboom, P., Emmerling, M., Fiebich, B.L., Finch, C.E., Frautschy, S., Griffin, W.S., Hampel, H., Hull, M., Landreth, G., Lue, L., Mrak, R., Mackenzie, I.R., McGeer, P.L., O'Banion, M.K., Pachter, J., Pasinetti, G., Plata-Salaman, C., Rogers, J., Rydel, R., Shen, Y., Streit, W., Strohmeyer, R., Tooyoma, I., Van Muiswinkel, F.L., Veerhuis, R., Walker, D., Webster, S., Wegrzyniak, B., Wenk, G., Wyss-Coray, T., 2000. Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383-421.

Bannwarth, B., Lapicque, F., Pehourcq, F., Gillet, P., Schaeverbeke, T., Laborde, C., Dehais, J., Gaucher, A., Netter, P., 1995. Stereoselective disposition of ibuprofen enantiomers in human cerebrospinal fluid. Br. J. Clin. Pharmacol. 40, 266-269.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Eikelenboom, P., Bate, C., Van Gool, W.A., Hoozemans, J.J., Rozemuller, J.M., Veerhuis, R., Williams, A., 2002. Neuroinflammation in Alzheimer's disease and prion disease. Glia 40, 232-239.

Eriksen, J.L., Sagi. S.A., Smith, T.E., Weggen, S., Das. P., McLendon. D.C., Ozols, V.V., Jessing, K.W., Zavitz, K.H., Koo, E.H., Golde, T.E., 2003. NSAIDs and enantiomers of flurbiprofen target γ-secretase and lower Aß42 in vivo. J. Clin. Invest. 112, 440-449.

Forloni, G., Colombo, L., Girola, L., Tagliavini, F., Salmona, M., 2001. Anti-amyloidogenic activity of tetracyclines: studies in vitro. F. E. B. S. Lett. 487, 404-407.

Gao, H.M., Liu, B., Zhang, W., Hong, J.S., 2003.   Novel anti-inflammatory therapy for Parkinson's disease.   Trends Pharmacol. Sci. 24, 395-401.

Gong, Y., Chang, L., Viola, K.L., Lacor, P.N., Lambert, M.P., Finch, C.E., Krafft, G.A., Klein, W.L., 2003.   Alzheimer's disease-affected brain: Presence of oligomeric Aß ligands (ADDLs) suggests a molecular basis for reversible memory loss.   Proc. Natl. Acad. Sci. U. S. A. 100, 10417-10422.

Hasegawa, K., Yamaguchi, I., Omata, S., Gejyo, F., Naiki, H., 1999.   Interaction between Aß(1-42) and Aß(1-40) in Alzheimer's ß-amyloid fibril formation in vitro.   Biochemistry 38, 15514-15521.

Hasegawa, K., Ono, K., Yamada, M., Naiki, H., 2002.   Kinetic modeling and determination of reaction constants of Alzheimer's ß-amyloid fibril extension and dissociation using surface plasmon resonance.   Biochemistry 41, 13489-13498.

in 't Veld, B.A., Launer, L.J., Hoes, A.W., Ott, A., Hofman, A., Breteler, M.M., Stricker, B.H., 1998.   NSAIDs and incident Alzheimer's disease. The Rotterdam Study.   Neurobiol. Aging 19, 607-611.

in t' Veld, B.A., Ruitenberg, A., Hofman, A., Launer, L.J., van Duijn, C.M., Stijnen, T., Breteler, M.M., Stricker, B.H., 2001.   Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N. Engl. J. Med. 345, 1515-1521.

Jarrett, J.T., Lansbury, P.T. Jr., 1993.   Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie?   Cell 73, 1055-1058.

Kisilevsky, R., Lemieux, L.J., Fraser, P.E., Kong, X., Hultin, P.G., Szarek, W.A., 1995.   Arresting amyloidosis in vivo using small-molecule anionic sulphonates or sulphates: implications for Alzheimer's disease.   Nat. Med. 1, 143-148.

Li, J., Zhu, M., Manning-Bog, A.B., Di Monte, D.A., Fink, A.L., 2004.   Dopamine and L-dopa

disaggregate amyloid fibrils: implications for Parkinson's and Alzheimer's disease.   F.A.S.E.B.J. 9, 962-964.

Lim, G.P., Yang, F., Chu, T., Chen, P., Beech, W., Teter, B., Tran, T., Ubeda, O., Ashe, K.H., Frautschy, S.A., Cole, G.M., 2000.   Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease.   J. Neurosci. 20, 5709-5714.

Lim, G.P., Yang, F., Chu, T., Gahtan, E., Ubeda, O., Beech, W., Overmier, J.B., Hsiao-Ashec, K., Frautschy, S.A., Cole, G.M., 2001.   Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice.   Neurobiol. Aging 22, 983-991.

McGeer, P.L., McGeer, E.G., 1995.   The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases.   Brain. Res. Brain. Res. Rev. 21, 195-218.

McGeer, P.L., Schulzer, M., McGeer, E.G., 1996.   Arthritis and anti-inflammatiry agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies.   Neurology 47, 425-432.

Naiki, H., Gejyo, F., 1999.   Kinetic analysis of amyloid fibril formation.   Methods Enzymol. 309, 305-18.

Naiki, H., Hasegawa, K., Yamaguchi, I., Nakamura, H., Gejyo, F., Nakakuki, K., 1998.   Apolipoprotein E and antioxidants have different mechanisms of inhibiting Alzheimer's ß-amyloid fibril formation in vitro.   Biochemistry 37, 17882-17889.

Naiki, H., Nakakuki, K., 1996.   First-order kinetic model of Alzheimer's ß-amyloid fibril extension in vitro.   Lab. Invest. 74, 374-383.

Ono, K., Hasegawa, K., Yamada, M., Naiki, H., 2002a.   Nicotine breaks down preformed Alzheimer's ß-amyloid fibrils in vitro.   Biol. Psychiatry 52, 880-886.

Ono, K., Hasegawa, K., Yoshiike, Y., Takashima, A., Yamada, M., Naiki, H., 2002b. Nordihydroguaiaretic acid potently breaks down pre-formed Alzheimer's ß-amyloid fibrils in vitro. J. Neurochem. 81, 434-440.

Ono, K., Yoshiike, Y., Takashima, A., Hasegawa, K., Naiki, H., Yamada, M., 2003.   Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease.   J. Neurochem. 87, 172-181.

Ono, K., Hasegawa, K., Naiki, H., Yamada, M., 2004a.   Anti-amyloidogenic activity of tannic acid and its activity to destabilize Alzheimer's ß-amyloid fibrils in vitro.   Biochim. Biophys. Acta 1690, 193-202.

Ono, K., Hasegawa, K., Naiki, H., Yamada, M., 2004b.   Curcumin has potent anti-amyloidogenic effects for Alzheimer's ß-amyloid fibrils in vitro.   J. Neurosci. Res. 75, 742-750.

Pappolla, M., Bozner, P., Soto, C., Shao, H., Robakis, N.K., Zagorski, M., Frangione, B., Ghiso, J., 1998.   Inhibition of Alzheimer ß-fibrillogenesis by melatonin.   J. Biol. Chem. 273, 7185-7188.

Pike, C.J., Walencewicz-Wasserman, A.J., Kosmoski, J., Cribbs, D.H., Glabe, C.G., Cotman, C.W., 1995.   Structure-activity analyses of ß-amyloid peptides: contributions of the ß25-35 region to aggregation and neurotoxicity.   J. Neurochem. 64, 253-265.

Rogers, J., Webster, S., Lue, L.F., Brachova, L., Civin, W.H., Emmerling, M., Shivers, B., Walker, D., McGeer, P., 1996.   Inflammation and Alzheimer's disease pathogenesis.   Neurobiol. Aging 17, 681-686.

Sagi, S.A., Weggen, S., Eriksen, J., Golde, T.E., Koo, E.H., 2003.   The non-cyclooxygenase targets of

non-steroidal anti-inflammatory drugs, lipoxygenases, peroxisome proliferator-activated receptor,

inhibitor of κB kinase, and NF κB, do not reduce amyloid ß42 production.   J. Biol. Chem. 278,

31825-31830.

Selkoe D.J., 2001.   Alzheimer's disease: genes, proteins, and therapy.   Physiol. Rev. 81, 741-766.

Soto, C., Kindy, M.S., Baumann, M., Frangione, B., 1996.   Inhibition of Alzheimer's amyloidosis by

peptides that prevent ß-sheet conformation.   Biochem. Biophys. Res. Commun. 226, 672-680.

Takahashi, Y., Hayashi, I., Tominari, Y., Rikimaru, K., Morohashi. Y., Kan, T., Natsugari, H.,

Fukuyama, T., Tomita, T., Iwatsubo, T., 2003.   Sulindac sulfide is a noncompetitive γ-secretase

inhibitor that preferentially reduces Aß42 generation.   J. Biol. Chem. 278, 18664-18670.

Thomas, T., Nadackal, T.G., Thomas, K., 2001.   Aspirin and non-steroidal anti-inflammatory drugs

inhibit amyloid-ß aggregation.   NeuroReport 12, 3263-3267.

Tomiyama, T., Shoji, A., Kataoka, K., Suwa, Y., Asano, S., Kaneko, H., Endo, N., 1996.   Inhibition of

amyloid ß protein aggregation and neurotoxicity by rifampicin.   Its possible function as a

hydroxyl radical scavenger.   J. Biol. Chem. 271, 6839-6844.

Weggen, S., Eriksen, J.L., Das, P., Sagi, S.A., Wang, R., Pietrzik, C.U., Findlay, K.A., Smith, T.E.,

Murphy, M.P., Bulter, T., Kang, D.E., Marquez-Sterling, N., Golde, T.E., Koo, E.H., 2001.   A

subset of NSAIDs lower amyloidogenic Aß42 independently of cyclooxygenase activity.   Nature

414, 212-6.

Yang, F., Lim, G.P., Begum, A.N., Ubeda, O.J., Simmons, M.R., Ambegaokar, S.S., Chen, P.P., Kayed,

R., Glabe, C.G., Frautschy, S.A., Cole, G.M., 2005.   Curcumin inhibits formation of amyloid beta

oligomers and fibrils, binds plaques, and reduces amyloid in vivo.   J. Biol. Chem. 280,

5892-5901.

Zeng, H., Zhang, Y., Peng, L., Shao, H., Menon, N.K., Yang, J., Salomon, A.R., Freidland, R.P.,

Zagorski, M.G., 2001.   Nicotine and amyloid formation.   Biol. Psychiatry 49, 248-257.

Zhou, Y., Su, Y., Li, B., Liu, F., Ryder, J.W., Wu, X., Gonzalez-DeWhitt, P.A., Gelfanova, V., Hale,

J.E., May, P.C., Paul, S.M., Ni, B., 2003.   Nonsteroidal anti-inflammatory drugs can lower

amyloidogenic Aß42 by inhibiting Rho.   Science 302, 1215-1217.

Legends for figures

Fig. 1.   Effects of Ibu (A, B), Ket (C), and Nap (D) on the kinetics of fAß(1-40) (A, C, D) and

fAß(1-42) (B) formation from fresh Aß(1-40) and Aß(1-42), respectively.   The reaction mixtures

containing 50 µM Aß(1-40) (A, C, D) or 25 µM Aß(1-42) (B), 50 mM phosphate buffer, pH 7.5, 100

mM NaCl, and 0 (●), 10 (○), or 50 µM (□) of Ibu (A, B), Ket (C), or Nap (D), were incubated at

37°C for the indicated times.   Each point represents the mean of three 5 µL aliquots from the same

sample.   Each figure is a representative pattern of 3 independent experiments.

Fig. 2.   Effects of Ibu (A, B), Ket (C), and Nap (D) on the kinetics of fAß(1-40) (A, C, D) and

fAß(1-42) (B) extension.   The reaction mixtures containing 10 µg/mL (2.3 µM) sonicated fAß(1-40)

(A, C, D) or fAß(1-42) (B), 50 µM Aß(1-40) (A, C, D) or Aß(1-42) (B), 50 mM phosphate buffer, pH

7.5, 100 mM NaCl, and 0 (●), 10 (○) or 50 µM (□) of Ibu (A, B), Ket (C), or Nap (D), were

incubated at 37°C for the indicated times.   Each point represents the mean of three 5 µL aliquots from

the same sample.   Each figure is a representative pattern of 3 independent experiments.   (E) Effect of

Aß(1-40) concentration on the initial rate of fAß(1-40) extension in the presence (○) and absence (●)

of Ibu.   The reaction mixtures containing 10 µg/mL (2.3 µM) sonicated fAß(1-40), 50 mM phosphate

buffer, pH 7.5, 100 mM NaCl, 0 (●) or 10 µM (○) Ibu, and 0, 10, 20, 30, 40, and 50 µM Aß(1-40),

were incubated at 37°C for 1 h.   Points represent means of three independent experiments.   In all

points, standard errors were within symbols.   Liner least-square fit was performed for each straight line

(R^2 = 0.971 and 0.959 for ○ and ●, respectively).

Fig. 3.   Electron micrographs of extended fAß(1-40).   The reaction mixtures containing 10 µg/mL

(2.3 µM) fAß(1-40), 50 µM Aß(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (B) or

50 µM Ibu (A, C), were incubated at 37°C for 0 (A), or 6 h (B, C).   Scale bars indicate a length of 250

nm.

Fig. 4.   Effects of Ibu (A,B), Ket (C), and Nap (D) on the kinetics of fAß(1-40) (A, C, D) and

fAß(1-42) (B) destabilization.   The reaction mixtures containing 25 µM fAß(1-40) (A, C, D) or

fAß(1-42) (B), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (●), 10 (○), or 50 µM (□) of

Ibu (A, B), Ket (C), or Nap (D), were incubated at 37°C for the indicated times.   Each point represents

the mean of three 5 µL aliquots from the same sample.   Each figure is a representative pattern of 3

independent experiments.

Fig. 5.   Electron micrographs of destabilized fAß(1-40).   The reaction mixtures containing 25 µM

fAß(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 50 µM Ibu was incubated at 37°C for

0 (A), 1 (B), or 6 h (C).   Scale bars indicate a length of 250 nm.

Fig. 6.   Effects of NSAIDs on the formation of fAß(1-40) (A) and fAß(1-42) (B) from fresh

Aß(1-40) and Aß(1-42), respectively, and the destabilization of fAß(1-40) (C) and fAß(1-42) (D).   In

(A) and (B), the reaction mixture containing 50 µM Aß(1-40) or 25 µM Aß(1-42), 50 mM phosphate

buffer, pH 7.5, 100 mM NaCl, and 50 µM NSAIDs was incubated at 37°C for 7 days and 24 h,

respectively.   In (C) and (D), the reaction mixture containing 25 μM fAß(1-40) or fAß(1-42), 50 mM

phosphate buffer, pH 7.5, 100 mM NaCl, and 50 μM NSAIDs was incubated at 37°C for 24 h.   Each

column represents the average of three independent experiments.   SD is indicated by bars.   N.S.

denotes not significant.   Significant difference ($p < 0.05$) was observed in all other combinations

(one-way analysis of variance, post-hoc test by Scheffe).

Fig. 7.   Dose-dependent inhibition of the formation of fAß(1-40) (A) and fAß(1-42) (B) from fresh

Aß(1-40) and Aß(1-42), respectively, and dose-dependent destabilization of fAß(1-40) (C) and

fAß(1-42) (D).   In (A) and (B), the reaction mixture containing 50 μM Aß(1-40) or 25 μM Aß(1-42),

50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0, 0.01, 0.1, 1, 10 and 50 μM NDGA (●), Rif

(■), SSide (□), Ibu (○), or nicotine (◆) were incubated at 37°C for 4 days or 24 h, respectively.   In

(C) and (D), the reaction mixture containing 25 μM fAß(1-40) or fAß(1-42), 50 mM phosphate buffer,

pH 7.5, 100 mM NaCl, and 0, 0.01, 0.1, 1, 10 and 50 μM NDGA (●), Rif (■), SSide (□), Ibu (○), or

nicotine (◆) were incubated at 37°C for 6 h.   Each point represents the average of three independent

experiments.   SD is indicated by bars.   The average without compounds was regarded as 100%.

Note that the final equilibrium levels in the presence of 50 μM SSide and Ibu were different from those

in Fig. 6 because different lots of Aß(1-40) and Aß(1-42) (530108 and 530914, respectively) were used

in these experiments.

Fig. 8.   Structures of NSAIDs examined in this study.
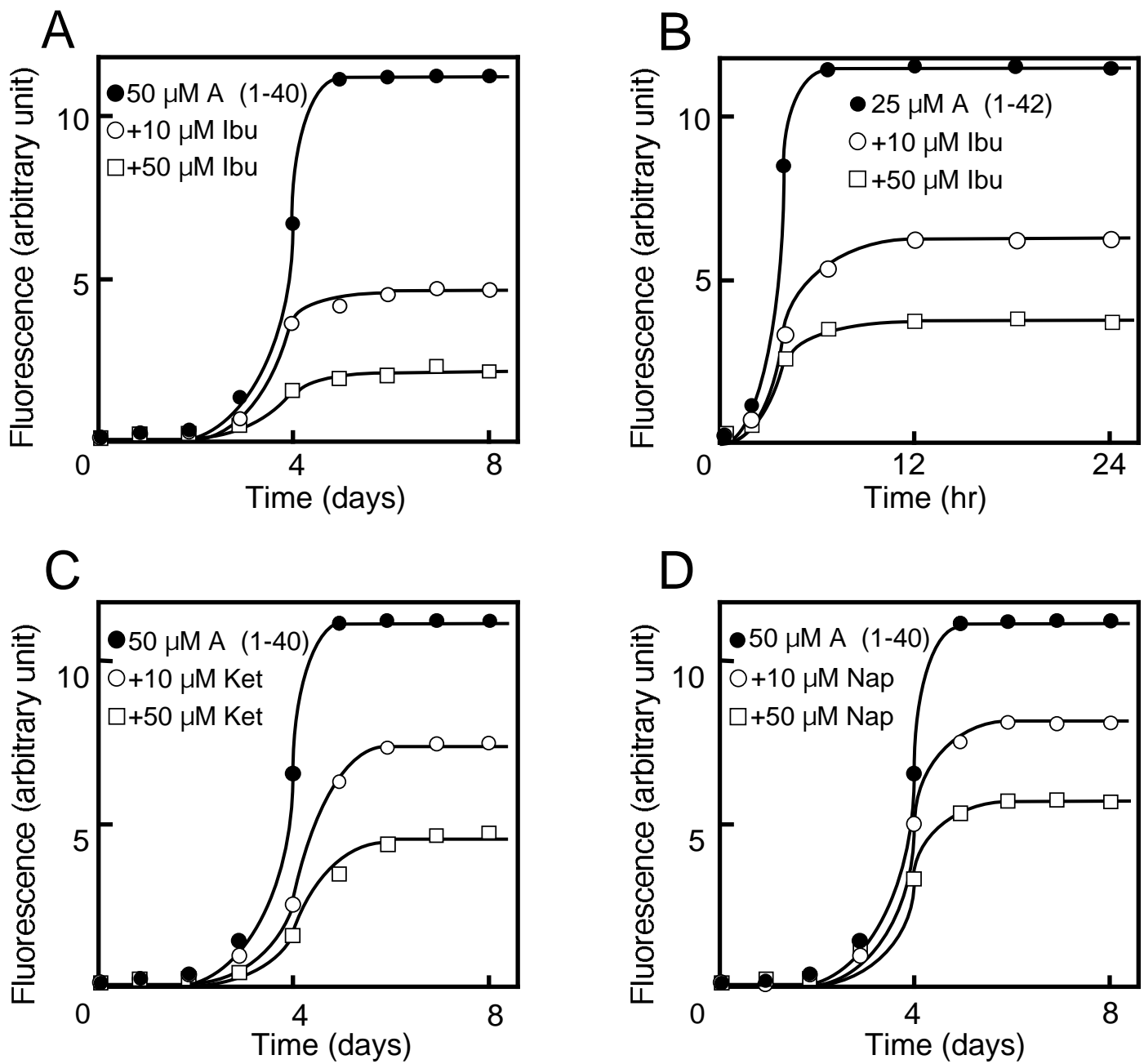
Fig. 1.
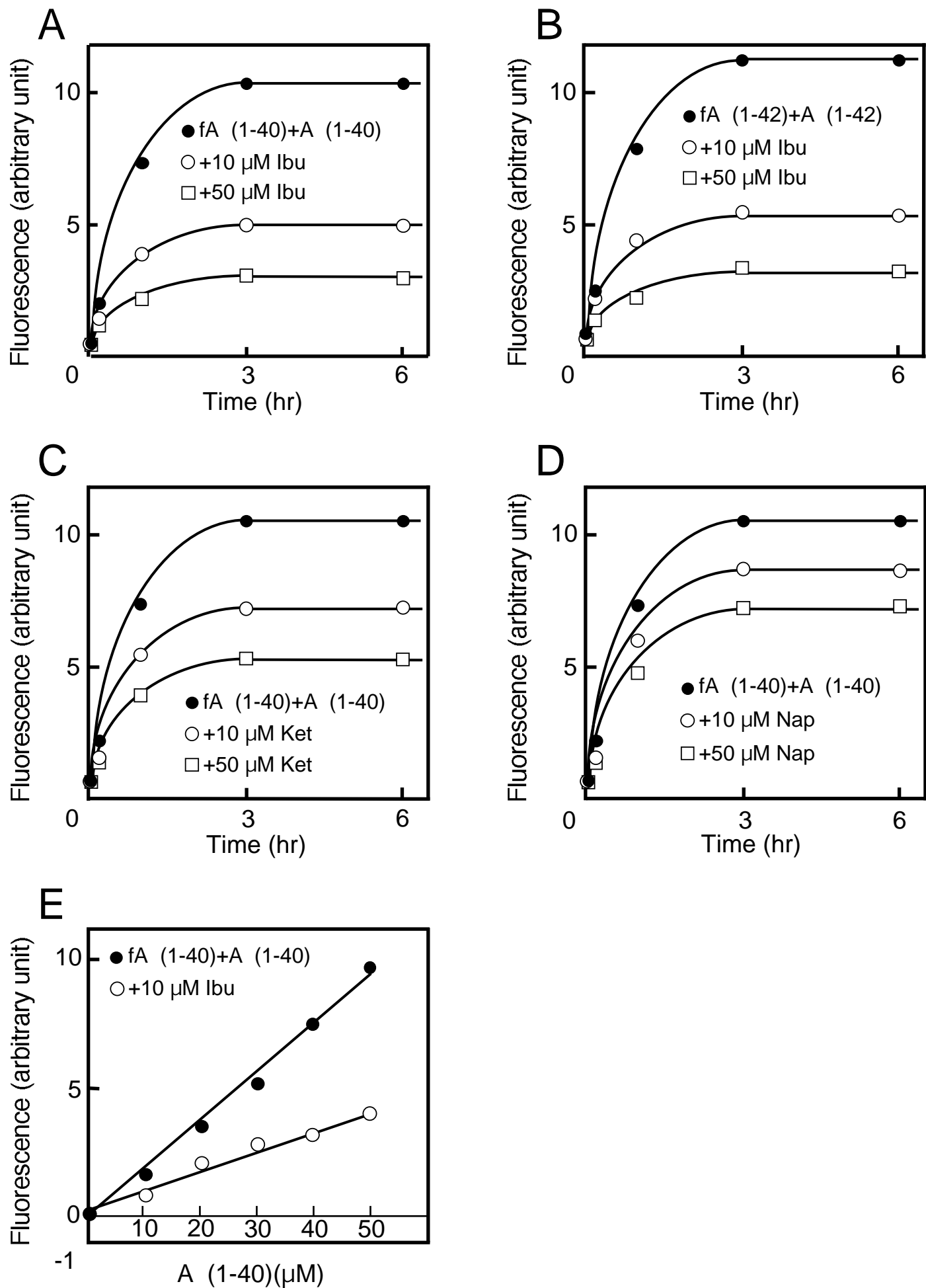
Fig. 2.

A
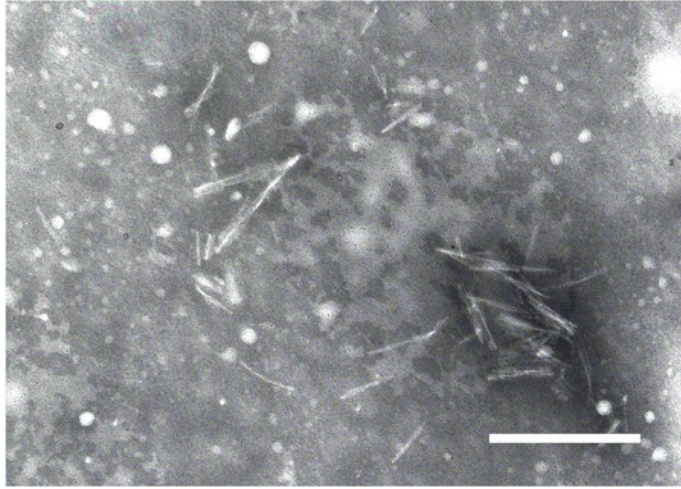
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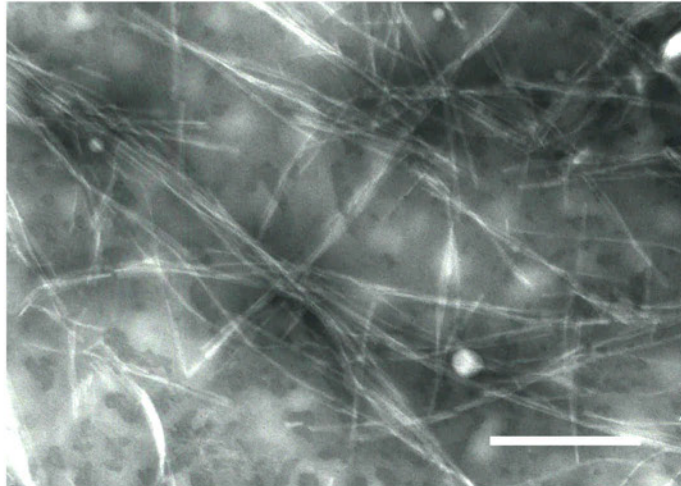
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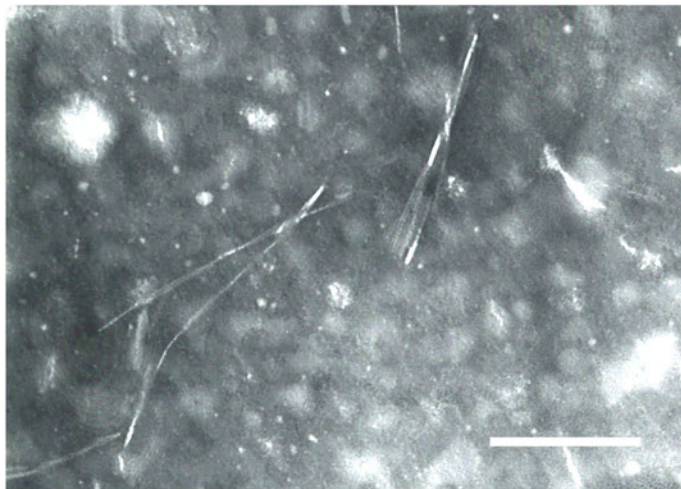
Fig. 3

Fig. 4.

A

B

C

Fig. 5

Fig. 6.

Fig. 7.

Ibuprofen (Ibu)

Aspirin (Asp)

Meclofenac acid (Mec)

Diclofenac acid (Dic)

Ketoprofen (Ket)

Flurbiprofen (Flu)

Naproxen (Nap)

Sulindac sulfide (SSide)

Indomethacin (Ind)

Fig. 8.