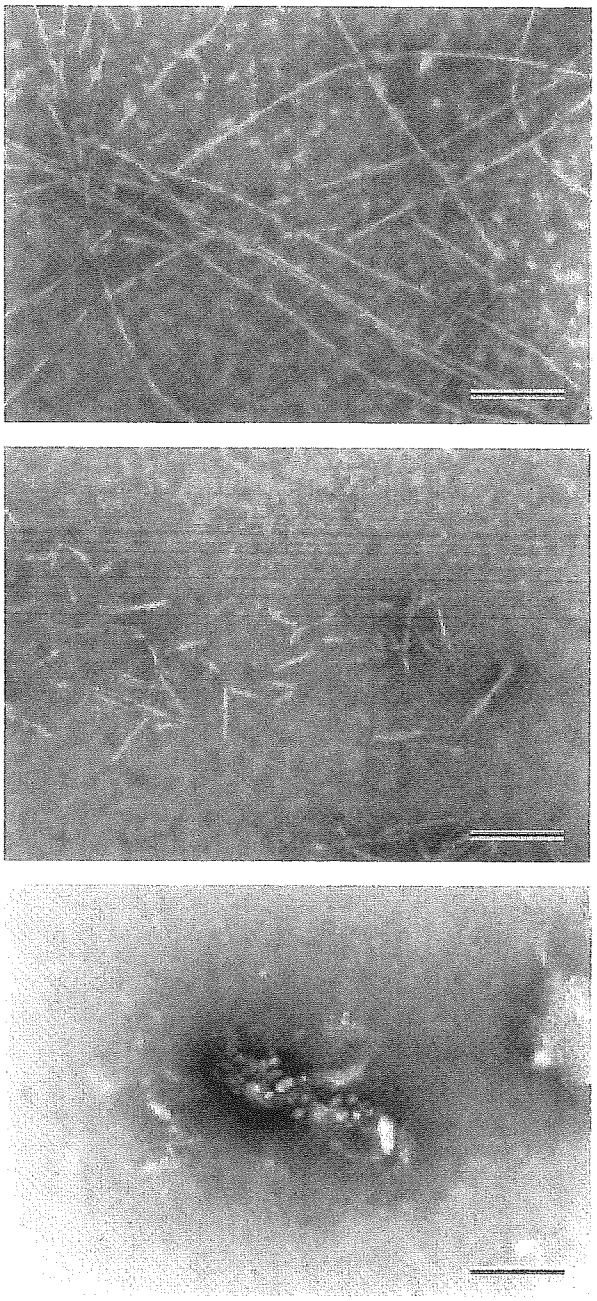

フレッシュなAβの線維は幅約10 nm，200 nm周期のらせん構造を示している（A）．ミリセチンとの反応開始1時間後には，短く切断された線維が多数観察された（B）．さらに6時間経過すると，無定形な凝集物が多数観察された（C）．スケールバー＝250 nm

図4　fAβ(1-40）分解反応の電顕写真

たところ，すでに形成されたAβ沈着も減少させた[1]．

クルクミンに関しては，10か月齢ADモデルマウスに6か月間，クルクミン160 ppmを投与すると，炎症反応を抑えるばかりでなく，脳内のAβ沈着も抑制させた[8]．

ニコチンについては，9か月齢ADモデルマウスに5.5か月間，ニコチン溶液200 μg/mlを投与すると脳内のAβ沈着を抑制させた[12]．

メラトニンについては，4か月齢ADモデルマウスにメラトニン溶液0.5 mg/mlを経口摂取させたところ，9.5か月齢以降において脳内のAβレベルの増加を抑制した[10]．また，Aβの抑制に伴って脳内のメラトニンレベルも増加していることも同時に確認された[10]．

Ⅳ．臨床試験にて効果が報告されている化合物

臨床試験においても効果が報告されている化合物として，リファンピシン[9]，ポリ硫酸化合物[2]，クリオキノール[17]などがある（表1）．

軽度および中等度のAD患者101人に対して3か月間，リファンピシン300 mg/日，ドキシサイクリン200 mg/日投与を行ったところ，認知機能において有意な効果を認めたことが報告された[9]．リファンピシンはクラミジア肺炎に対する治療に用いられるが，クラミジア抗原やクラミジア抗体に関しては有意な変化は認めなかった[9]．

ポリ硫酸化合物の*in vitro*および*in vivo*実験系におけるfAβ形成抑制作用が報告されているが[6]，最近ポリ硫酸化合物NC-531（Alzhemed®）による臨床試験が行われている[2]．AD患者58人に対して，NC-531を200または300 mg/日を投与するphase Ⅱ試験を行い，約70%の軽度AD患者において投与20か月後に認知機能の維持または改善を認めたことが報告された[2]．さらにphase Ⅲ試験が進行中である[2]．

クリオキノールの臨床試験では，AD患者36人に対して内服投与を行ったところ，とくにより重度な患者において有意な効果を認めたという（phase Ⅱ）[17]．

おわりに

Aβ凝集抑制薬候補として，*in vitro* だけでなく，*in vivo* 実験系，さらには臨床試験においても効果を有する化合物が報告されてきており，将来のAD治療において重要な位置を占める可能性がある．

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痴呆症診療のための実践的教育企画

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〈要　約〉痴呆症の診断方法だけでなく的確な治療・ケアの方法まで踏み込み，模擬患者を使った問診，診察のロールプレーイングにより，明日からの日常臨床に応用できるように配慮した点が大きな特徴である．約3時間にわたりビデオ上映を交えたポイント解説と，問診・神経学的診察・画像診断の実演を行った．

Key words：早期発見の意義，アルツハイマー型痴呆の鑑別，診断のロールプレーイング，薬物療法，告知とそのプロセス

（日老医誌　2005；42：409—410）

痴呆症早期発見の意義

早期発見・早期治療の意義として●治療可能な痴呆症をみつける　●アルツハイマー型痴呆であれば薬物治療により，症状の改善や症状の進行を遅らせることができる　●治療，生活に関して患者自身が自己決定できる　●介護や生活の計画が余裕を持って立てられることなどがある．

アルツハイマー型痴呆の診断の考え方

DSM-IV（米国精神医学診断統計便覧第4版）とNINCDS-ADRDAによる診断基準を解説した．特にアルツハイマー型痴呆の診断として「症状」「経過」「鑑別」が重要である．

痴呆症の中核症状についてのアセスメント

中核症状について具体的な実例をビデオで供覧した後，記憶障害，見当識障害，判断・実行機能障害，失語・失行・失認の具体的症状と診断のポイント，アルツハイマー型痴呆の経過に関する解説を行った．

アルツハイマー型痴呆の鑑別

痴呆と間違えられやすいせん妄とうつ病の具体的症状およびその特徴を理解することの意義をビデオを交えて説明した．また，痴呆を呈するその他の疾患の同定手順（神経学的診察，尿検査・血液検査，画像検査）に関する概要を説明した．

神経学的診察

痴呆の原因の鑑別として神経学的所見（構音障害・嚥下障害，麻痺，筋固縮（強剛），振戦，深部腱反射亢進，歩行障害）の重要性を説明した．専門医でなくても可能な簡単に施行可能な神経所見の取り方をビデオ上映を交えながら具体的に解説した．具体的には，診察室へ入室される際の歩行状態，バレーサイン，振戦，筋固縮，構成行為の診かたであり，特殊な診察道具を用いずに施行可能なものである．

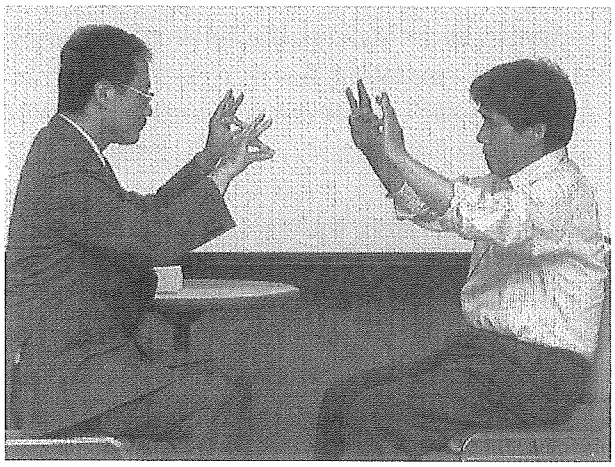

図1　講師が模擬患者さんを相手にロールプレイを行っているところ

Practical educational program for management of dementia

1) Akira Honma：東京都老人総合研究所
2) Kastuya Urakami：鳥取大学医学部保健学科生体制御学講座
3) Shin Kitamura：日本医科大学付属第二病院内科
4) Masahito Yamada：金沢大学医学部神経内科学
5) Masahiro Shigeta：東京都立保健科学大学大学院保健科学研究科

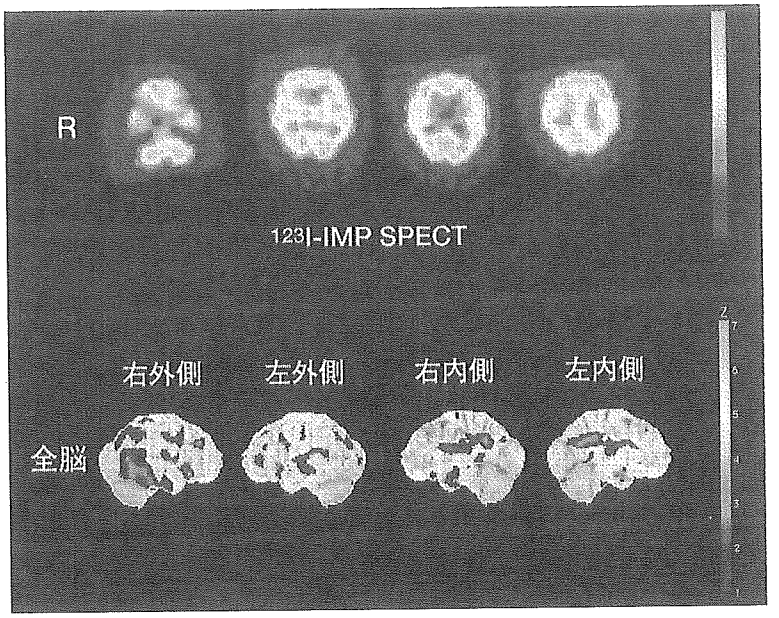

図2　アルツハイマー病患者さんの3D-SSP所見

画像所見の読み方

アルツハイマー型痴呆と脳血管性痴呆の主な鑑別点の解説と，慢性硬膜下血腫，脳腫瘍，正常圧水頭症，軽度アルツハイマー型痴呆の画像診断のポイントを説明した．アルツハイマー型痴呆と脳血管性痴呆の2症例の問診風景のビデオを上映した後，疑われる疾患を参加者に質問しながら，問診と画像診断の注意点を確認した．

痴呆症診断のロールプレーイング

本セミナーの大きな特徴とも言えるのが，臨床ですぐに活かせる診断技術の習得を目的としたロールプレーイングである．講師が問診と診察のロールプレーイングを行い，そのポイントを説明した後，2症例について参加者によるロールプレーイングを行い，その様子を確認しながら問診，構成行為などの神経学的所見，画像所見の注意点についての解説をした．

問診では患者に「来院方法」「最近した検査について」「今日の年月日」「最近の様子」「趣味」などを聞き，最近の記憶の障害，時間見当識や病識の有無，失語等の状況をチェックした．神経学的診察では，麻痺や筋固縮の有無，手指を使った簡単な構成行為を真似られるかどうかを調べた（図1）．またアルツハイマー型痴呆の画像診断のポイントとして，MRIでは側頭葉内側の萎縮などが，SPECTでは両側側頭葉外側や頭頂葉外側，後部帯状回などでの血流低下の有無の重要性を説明した（図2）．

アルツハイマー型痴呆に対する薬物療法

塩酸ドネペジルによる治療の効果，服用の仕方，副作用などを患者や家族にいかに説明するか，効果判定のポイント，治療開始時期，長期投与の注意点などについて解説した．

告知とそのプロセス

検査結果や生活上の注意点，診断，今後の注意などについての説明は，丁寧に段階を経て行うことが患者や家族との信頼関係を築く上で重要であるか，その意義についてビデオ上映を交えて解説した．

地域の中でのケアシステム

宮城県栗原郡での取り組みをビデオで紹介し，医療・福祉の連携体制の重要性を説明した．専門的知識を持つかかりつけ医が医療・介護の両方の現場をサポートする上で大きな役割を果たすことの理解を促した．

ま と め

用語のみでは難しい病態の理解をビデオなどの画像を使い，また問診や神経所見のとり方を模擬患者さんを使ってロールプレーイングを行ったため，明日からの診療に役立てれる内容と参加者から評価を頂いた．この企画をとうして今後のアルツハイマー型痴呆診療に役立ててもらうことを期待し，本実践的教育企画を終えた．

HIGH LIGHT CME

Diagnosis and management of dementia with Lewy bodies

Third report of the DLB consortium

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Abstract—The dementia with Lewy bodies (DLB) Consortium has revised criteria for the clinical and pathologic diagnosis of DLB incorporating new information about the core clinical features and suggesting improved methods to assess them. REM sleep behavior disorder, severe neuroleptic sensitivity, and reduced striatal dopamine transporter activity on functional neuroimaging are given greater diagnostic weighting as features suggestive of a DLB diagnosis. The 1-year rule distinguishing between DLB and Parkinson disease with dementia may be difficult to apply in clinical settings and in such cases the term most appropriate to each individual patient should be used. Generic terms such as Lewy body (LB) disease are often helpful. The authors propose a new scheme for the pathologic assessment of LBs and Lewy neurites (LN) using alpha-synuclein immunohistochemistry and semiquantitative grading of lesion density, with the pattern of regional involvement being more important than total LB count. The new criteria take into account both Lewy-related and Alzheimer disease (AD)-type pathology to allocate a probability that these are associated with the clinical DLB syndrome. Finally, the authors suggest patient management guidelines including the need for accurate diagnosis, a target symptom approach, and use of appropriate outcome measures. There is limited evidence about specific interventions but available data suggest only a partial response of motor symptoms to levodopa: severe sensitivity to typical and atypical antipsychotics in ~50%, and improvements in attention, visual hallucinations, and sleep disorders with cholinesterase inhibitors.

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Clinical diagnostic criteria for DLB. Since the publication of Consensus criteria for clinical and pathologic diagnosis of dementia with Lewy bodies (DLB),[1,2] new information indicates that clinical criteria for probable DLB have acceptable specificity, but suboptimal sensitivity.[3,4] Reasons identified include difficulties in recognition of the core feature fluctuation[5,6] and a low rate of all core features (fluctuation, visual hallucinations, parkinsonism) in the presence of neocortical, neurofibrillary tangle (NFT) pathology.[7-9] The criteria have therefore been modified (table 1) to incorporate additional items indicative of LB pathology. Distinction is made between clinical features or investigations that are *suggestive* of DLB, i.e., have been demonstrated to be significantly more frequent than in

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*Author affiliations are listed in the appendix at the end of this article. Members of the Consortium on DLB are listed in appendix E-1 at www.neurology.org.

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Table 1 Revised criteria for the clinical diagnosis of dementia with Lewy bodies (DLB)

1. *Central feature* (essential for a diagnosis of possible or probable DLB)

   Dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function. Prominent or persistent memory impairment may not necessarily occur in the early stages but is usually evident with progression. Deficits on tests of attention, executive function, and visuospatial ability may be especially prominent.

2. *Core* features (two core features are sufficient for a diagnosis of probable DLB, one for possible DLB)

   Fluctuating cognition with pronounced variations in attention and alertness

   Recurrent visual hallucinations that are typically well formed and detailed

   Spontaneous features of parkinsonism

3. *Suggestive features* (If one or more of these is present in the presence of one or more core features, a diagnosis of probable DLB can be made. In the absence of any core features, one or more suggestive features is sufficient for possible DLB. Probable DLB should not be diagnosed on the basis of suggestive features alone.)

   REM sleep behavior disorder

   Severe neuroleptic sensitivity

   Low dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET imaging

4. *Supportive features* (commonly present but not proven to have diagnostic specificity)

   Repeated falls and syncope

   Transient, unexplained loss of consciousness

   Severe autonomic dysfunction, e.g., orthostatic hypotension, urinary incontinence

   Hallucinations in other modalities

   Systematized delusions

   Depression

   Relative preservation of medial temporal lobe structures on CT/MRI scan

   Generalized low uptake on SPECT/PET perfusion scan with reduced occipital activity

   Abnormal (low uptake) MIBG myocardial scintigraphy

   Prominent slow wave activity on EEG with temporal lobe transient sharp waves

5. A diagnosis of DLB is *less likely*

   In the presence of cerebrovascular disease evident as focal neurologic signs or on brain imaging

   In the presence of any other physical illness or brain disorder sufficient to account in part or in total for the clinical picture

   If parkinsonism only appears for the first time at a stage of severe dementia

6. *Temporal sequence* of symptoms

   DLB should be diagnosed when dementia occurs before or concurrently with parkinsonism (if it is present). The term Parkinson disease dementia (PDD) should be used to describe dementia that occurs in the context of well-established Parkinson disease. In a practice setting the term that is most appropriate to the clinical situation should be used and generic terms such as LB disease are often helpful. In research studies in which distinction needs to be made between DLB and PDD, the existing 1-year rule between the onset of dementia and parkinsonism DLB continues to be recommended. Adoption of other time periods will simply confound data pooling or comparison between studies. In other research settings that may include clinicopathologic studies and clinical trials, both clinical phenotypes may be considered collectively under categories such as LB disease or alpha-synucleinopathy.

other dementing disorders, and *supportive* features, which commonly occur but with lower specificity. Clinicians should adopt a high index of suspicion, screening all patients with dementia for possible DLB (one core or one suggestive feature) paying particular attention to the early clinical presentation.[7,10] The widespread use of improved assessment tools and methods of investigation should further improve diagnostic accuracy.

Progressive disabling mental impairment is a mandatory requirement for the diagnosis of DLB. This statement[1] remains true although it is apparent that disability in DLB derives not only from cognitive impairment but also from neuropsychiatric, motoric, sleep, and autonomic dysfunction. The cognitive profile of DLB comprises both cortical and subcortical impairments with substantial attentional deficits and prominent executive and visuospatial dysfunction.[11,12] A "double discrimination" can help differentiate DLB from Alzheimer disease (AD), with relative preservation of confrontation naming and short and medium term recall as well as recognition, and greater impairment on verbal fluency, visual perception and performance tasks.[13-15] Patients with DLB with neocortical neurofibrillary tangles (NFTs) often lack this profile, showing pronounced memory deficits more characteristic of AD. Composite global cognitive assessment tools such as the Mini-Mental State Examination (MMSE) cannot be relied upon to distinguish DLB from other common dementia syndromes and some patients who meet criteria for DLB will score in the normal range.

DLB and dementia associated with Parkinson disease (PDD). Many patients with PD develop dementia, typically 10 years or more after onset of motor symptoms.[16,17] Other than age at onset, temporal course, and possibly levodopa responsivity,[18,19] no major differences between DLB and PDD have been found in any variable examined including cognitive profile,[20] attentional performance,[21] neuropsychiatric features,[22] sleep disorders,[23] autonomic dysfunction,[24] type and severity of parkinsonism,[25] neuroleptic sensitivity,[26] and responsiveness to cholinesterase inhibitors.[27,28] The relative contributions of LB formation and synuclein pathology, AD-type pathology, neuron loss, or neurochemical deficits as determinants of dementia in PD remain unresolved although recent studies suggest that Lewy-related pathology is more strongly associated than AD-type changes.[29-31]

The distinction between DLB and PDD as two distinct clinical phenotypes based solely on the temporal sequence of appearance of symptoms has been criticized by those who regard the different clinical presentations as simply representing different points on a common spectrum of LB disease, itself underpinned by abnormalities in alpha-synuclein metabolism. This unitary approach to classification may be preferable for molecular and genetic studies and for developing therapeutics. Descriptive labels that include consideration of the temporal course are preferred for clinical, operational definitions. DLB should be diagnosed when dementia occurs before or concurrently with parkinsonism and PDD should be used to describe dementia that occurs in the context of well-established PD. The appropriate term will depend upon the clinical situation and generic terms such as LB disease are often helpful. In research studies in which distinction is made between DLB and PDD, the 1-year rule between the onset of dementia and parkinsonism for DLB should be used. Adoption of other time periods will simply confound data pooling or comparison between studies. In other research settings including pathologic studies and clinical trials, both clinical phenotypes may be considered collectively under categories such as LB disease or alpha-synucleinopathy.

Core features. Although no major amendments to the three core features of DLB are proposed, improved methods for their clinical assessment are recommended for use in diagnosis and measurement of symptom severity.

Fluctuation. It is the evaluation of fluctuation that causes the greatest difficulty in clinical practice.[32] Inter-rater reliability is said to be low[5,6] although reports have generally been based upon review of pre-existing case records and notes, rather than direct rating of patients. Questions such as "are there episodes when his/her thinking seems quite clear and then becomes muddled?" were previously suggested as useful probes, but two recent studies[33,34] found 75% of both AD and DLB carers to respond positively. More detailed questioning and qualitative analysis of carers' replies is therefore needed. The Clinician Assessment of Fluctuation scale[35] requires an experienced clinician to judge the severity and frequency of "fluctuating confusion" or "impaired consciousness" over the previous month. The semi-structured One Day Fluctuation Assessment scale[35] can be administered by less experienced raters and generates a cut-off score to distinguish DLB from AD or vascular dementia (VaD). The Mayo Fluctuations Composite Scale[34] requires three or more "yes" responses from caregivers to structured questions about the presence of daytime drowsiness and lethargy, daytime sleep >2 hours, staring into space for long periods, or episodes of disorganized speech, as suggestive of DLB rather than AD. Recording variations in attentional performance using a computer based test system offers an independent method of measuring fluctuation, which is also sensitive to drug treatment effects.[36] Which of these various available methods is most appropriate will depend upon the setting and the level of expertise available. It is recommended that at least one formal measure of fluctuation is used when applying DLB diagnostic criteria and that staff are appropriately trained in its use.

Visual hallucinations. Recurrent, complex visual hallucinations (VH) continue to be one of the most useful signposts to a clinical diagnosis of DLB. They are generally present early in the course of illness with characteristics as described in the original report.[1] Informant-based assessment tools such as the Neuropsychiatric Inventory (NPI)[37] are helpful both for screening for VH and assessing their severity and frequency but do not always distinguish them from hallucinations in other sensory modalities. Caregivers tend to under-report VH and patients with mild to moderate cognitive impairment can contribute useful information about their presence and quality.[38] Patients with DLB with VH show more profound visuoperceptual dysfunction compared to those without hallucinations.[39,40] Increased numbers of LB in the anterior and inferior temporal lobe and amygdala at autopsy are associated with the presence and onset of VH,[41] each of these areas being implicated in the generation of complex visual images. Brain perfusion imaging demonstrates reduced occipital uptake[42,43] in areas identified as primary and secondary visual cortex.[44] VH are associated with greater deficits in cortical acetylcholine[45,46] and their presence may predict a good response to cholinergic therapy.[47]

Parkinsonism. The severity of extrapyramidal motor features in DLB is generally similar to that of age-matched patients with PD with or without dementia[26] with an average 10% annual progression rate.[48] There is an axial tendency with greater postural instability, gait difficulty, and facial immobility[49] than in non-demented patients with PD. Rest tremor is less common. The assessment of motor features may be complicated by the presence of cognitive impairment. A simple, five-item subscale of the Unified PD Rating Scale (UPDRS)[50,51] contains only those items that can reliably be assessed in DLB

independent of severity of dementia (tremor at rest, action tremor, body bradykinesia, facial expression, rigidity). Levodopa responsiveness in DLB[18,19] is almost certainly less than in uncomplicated PD, possibly because of intrinsic striatal degeneration[52] and the fact that a significant proportion of the parkinsonian symptoms may be non-dopaminergic in origin.

Suggestive features. If one or more of these is present, in addition to one or more core features, a diagnosis of probable DLB should be made. Possible DLB can be diagnosed if one or more suggestive features is present in a patient with dementia even in the absence of any core features. Suggestive features therefore have a similar diagnostic weighting as core clinical features but are not in the light of current knowledge considered sufficient, even in combination, to warrant a diagnosis of probable DLB in the absence of any core feature.

REM sleep behavior disorder. REM sleep behavior disorder (RBD) is manifested by vivid and often frightening dreams during REM sleep, but without muscle atonia. Patients therefore appear to "act out their dreams" vocalizing, flailing limbs, and moving around the bed sometimes violently. Vivid visual images are often reported, although the patient may have little recall of these episodes. The history is obtained from the bed partner, who may report many years of this sleep disorder prior to the onset of dementia and parkinsonism.[53] RBD is frequently associated with an underlying synucleinopathy—PD, DLB, or multiple system atrophy (MSA)—and only rarely with other neurodegenerative disorders.[54] Associated sleep disorders in DLB including excessive daytime drowsiness may also contribute to the fluctuating pattern. Screening questions about the presence of day and night time sleep disturbance should always be asked, facilitated by the use of sleep questionnaires, particularly those that query bed partners about a history of repeated episodes of "acting out dreams."[23] The diagnosis of RBD may be confirmed by polysomnography.

Severe neuroleptic sensitivity. Deliberate pharmacologic challenge with D2 receptor blocking agents should not be used as a diagnostic strategy for DLB because of the high morbidity and mortality associated with neuroleptic sensitivity reactions,[55] which are characterized by the acute onset or exacerbation of parkinsonism and impaired consciousness.[56] Approximately 50% of patients with DLB receiving typical or atypical antipsychotic agents do not react so adversely and a history of neuroleptic tolerance does not therefore exclude a diagnosis of DLB. A positive history of severe neuroleptic sensitivity is, by contrast, strongly suggestive of DLB.

Dopamine transporter imaging. Functional imaging of the dopamine transporter (DAT) defines integrity of the nigrostriatal dopaminergic system and currently has its main clinical application in assisting diagnosis of patients with tremor of uncertain etiology.[57] Imaging with specific ligands for DAT, e.g., FP-CIT, beta-CIT, IPT, TRODAT, provides a marker for presynaptic neuronal degeneration. DAT imaging is abnormal in idiopathic PD, MSA, and progressive supranuclear palsy (PSP). Low striatal DAT activity also occurs in DLB but is normal in AD,[58] making DAT scanning particularly useful in distinguishing between the two disorders.[59,60]

Supportive features. These are features (see table 1) that are commonly present in DLB but lack sufficient diagnostic specificity to be categorized as core or suggestive. Routine enquiry should be made about such symptoms since patients and carers may not consider them related to the dementing process. Severe autonomic dysfunction may occur early in disease, producing orthostatic hypotension, neurocardiovascular instability, urinary incontinence, constipation, and impotence, as well as eating and swallowing difficulties.[61-63] Autonomic dysfunction may also contribute to repeated falls and syncope and the transient losses of consciousness that are seen in some patients with DLB.[64] Systematized delusions, hallucinations in other modalities, and depression may all occur during the course of DLB and if they are prominent early, they can lead to diagnostic confusion with late onset psychosis, delusional depression, or other primary psychiatric diagnoses.[10,65]

Exclusion features. Careful exclusion of other systemic or neurologic disorders that may explain the clinical presentation is essential. Particular difficulty exists in relation to attributing clinical significance to evidence of cerebrovascular disease, since pathologic and imaging studies suggest that white matter lesions (periventricular and deep white matter), microvascular changes, and lacunes may be present in up to 30% of autopsy confirmed DLB cases.[66,67] A diagnosis of DLB with cerebrovascular disease may sometimes be the most appropriate.

Special investigations. A recent review concluded that there are as yet no clinically applicable genotypic or CSF markers to support a diagnosis of DLB.[3,68] The role of DAT transporter scanning has already been discussed. Other imaging investigations can also be helpful, including preservation of hippocampal and medial temporal lobe volume on MRI,[69,70] atrophy of the putamen,[71] and occipital hypoperfusion (SPECT) and hypometabolism (PET)[42,43,72-74] without occipital atrophy on MRI.[75] Other features such as the degree of generalized atrophy, rate of progressive brain atrophy, and severity of white matter lesions do not aid in differential diagnosis from other dementia subtypes.[76,77] Scintigraphy with [I-123] metaiodobenzyl guanidine (MIBG),[78] which enables the quantification of postganglionic sympathetic cardiac innervation, is reduced in DLB and has been suggested to have high sensitivity and specificity in the differential diagnosis from AD.[79] Confirmatory studies with larger patient numbers are required. The standard EEG may show early slowing, epoch by epoch fluctuation, and transient temporal slow wave activity.[3]

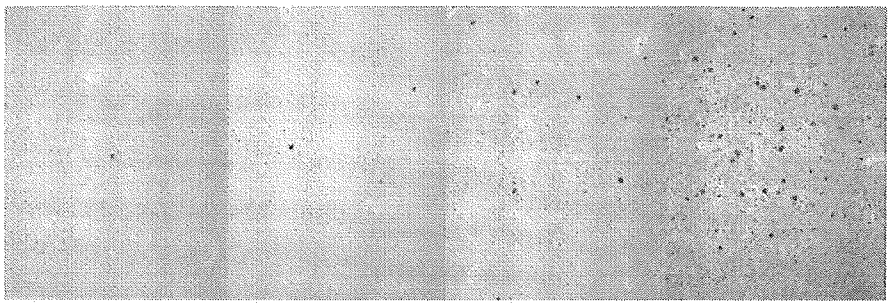

Figure. Staging of alpha-synuclein pathology in dementia with Lewy bodies (DLB). Alpha-synuclein immunostaining in cerebral cortex of DLB cases illustrating increasing severity of Lewy bodies (LBs) and LB pathology scored as stages 1 to 4. Stage 1 = sparse LBs or Lewy neurites (LNs); Stage 2 = >1 LB per high power field and sparse LNs; Stage 3 = ≥4 LBs and scattered LNs in low power field; Stage 4 = numerous LBs and LNs. Images courtesy of Dr. E. Jaros. 5 μm thick sections, pretreated with pressure cooker for 1 minute in EDTA pH8; Vector Elite Kit, Novocastra mouse monoclonal alpha-synuclein antibody (clone KM51), 1:30 dilution, DAB final reaction product.

Pathologic assessment and diagnostic criteria for DLB. *Dementia with Lewy bodies as a pathologic diagnostic category.* DLB was originally defined as a clinicopathologic entity with a specific constellation of clinical features, and a descriptive approach was proposed for assessing neuropathology.[1] The only neuropathologic requirement for DLB was the presence of LBs somewhere in the brain of a patient with a clinical history of dementia. Other pathologic features, e.g., senile plaques and neuron loss, could occur, but they were not inclusive or exclusive to the diagnosis. In many but not all cases the neuropathologic findings conform to those previously described as limbic or diffuse LB disease.[80,81] This liberal definition has the advantage of being widely inclusive, bringing many neuropathologic cases into consideration for DLB; however, as increasingly sensitive methods for detecting LBs have been developed, as many as 60% of AD cases may be considered to meet pathologic criteria for DLB using the 1996 criteria. Virtually none of these patients will have had the DLB clinical syndrome as described above, especially those cases with extensive NFTs[8,9] or those with one or more LBs in the amygdala but without significant Lewy-related pathology in other brain regions.[82] The inclusion of such cases as pathologically confirmed DLB has contributed to a view that the clinical criteria have suboptimal sensitivity.[4]

New recommendations are proposed that take into account both the extent of Lewy-related pathology and AD-type pathology in assessing the degree of certainty that the neuropathologic findings explain the DLB clinical syndrome. The scheme proposed should provide increased diagnostic specificity, since cases in which LBs are detected in the setting of extensive AD-type pathology that is likely to obscure the clinical features of the DLB syndrome are now classified as having a "low likelihood" of DLB.

Identification of Lewy bodies and Lewy-related pathology. LBs and Lewy neurites (LN) are pathologic aggregations of alpha-synuclein. They are also associated with intermediate filaments, chaperone proteins, and elements of the ubiquitin-proteasome system, indicating a role of the aggresomal response, but these features are not specific for LBs and are found in other neuronal inclusions.[83,84]

While hematoxylin and eosin (H&E) histologic staining may be adequate for detection of brainstem type LBs, it is not sufficient for cortical LBs and it is incapable of detecting LNs. Ubiquitin immunohistochemistry, which unequivocally stains LBs and LNs, can only be recommended in cases with minimal concurrent AD-type pathology, since ubiquitin is also present in NFTs, which can be easily confused with LBs. Rather than ubiquitin immunohistochemistry, it is now more appropriate to use immunohistochemical staining for alpha-synuclein, since this has been shown to be the most sensitive and specific method currently available for detecting LBs and Lewy-related pathology. We also recommend a semiquantitative grading of lesion density rather than the counting methods previously proposed (see figure).

Brain sampling and evaluation of Lewy-related pathology. The scheme previously proposed[1] for characterization of regional involvement of the brain with respect to LB pathology, i.e., brainstem, limbic, and diffuse cortical types, as well as the recommended tissue sampling procedures, remains unchanged. The previous Consortium protocol advised counting LB density in five cortical regions with a summed score for the overall LB rating. Given the poor inter-rater reliability of counting of LBs, the new recommendations propose a semiquantitative grading of severity of Lewy-related pathology into mild, moderate, severe, and very severe, along lines similar to those used to grade SP and NFTs by the CERAD protocol.

Brain sampling and evaluation of AD-type pathology in DLB. At the time of the original statement there was considerable uncertainty about the significance of coexisting AD-type pathology[85] and the most widely used method for evaluating AD-type pathology was the CERAD protocol.[86] Subsequently, a working group of the NIA-Reagan Institute expanded upon the CERAD protocol, which used a plaque-based diagnostic algorithm, by adding assessments of topographic stages of neurofibrillary pathology.[87] As well as adding NFTs to the diagnostic algorithm, the NIA-Reagan criteria admit that the

Table 2 Assignment of Lewy body type based upon pattern of Lewy-related pathology in brainstem, limbic, and neocortical regions

Lewy body type pathology	Brainstem regions			Basal forebrain/limbic regions				Neocortical regions		
	IX-X	LC	SN	nbM	Amygdala	Transentorhinal	Cingulate	Temporal	Frontal	Parietal
Brainstem-predominant	1-3	1-3	1-3	0-2	0-2	0-1	0-1	0	0	0
Limbic (transitional)	1-3	1-3	1-3	2-3	2-3	1-3	1-3	0-2	0-1	0
Diffuse neocortical	1-3	1-3	1-3	2-3	3-4	2-4	2-4	2-3	1-3	0-2

Brain regions are as defined anatomically in the original Consensus report.[1]

IX = 9th cranial nerve nucleus; X = 10th cranial nerve nucleus; LC = locus ceruleus; SN = substantia nigra; nbM = nucleus basalis of Meynert.

fit between clinical and pathologic features is imperfect and that the best that can be accomplished at present is a probability statement about the likelihood that the neuropathologic findings account for dementia. This approach has been adopted in the proposed DLB criteria which assess the likelihood that the neuropathologic findings predict the clinical syndrome of DLB. The likelihood that the observed neuropathology explains the DLB clinical syndrome is *directly related to the severity of Lewy-related pathology, and inversely related to the severity of concurrent AD-type pathology.* This approach is based on studies that demonstrate that clinical diagnostic accuracy for DLB is higher in patients with low burdens of AD-type pathology.[7,8,88] This revision is prompted by the body of literature that deals with clinicopathologic correlations in DLB and the desire to implement more rigorous and specific neuropathologic criteria than currently exist. The proposal obviously requires further research to test its validity. The proposal can be summarized as follows.

- Cases should be assigned a likelihood that the dementia can be attributed to AD pathology using the NIA-Reagan criteria, which employs the CERAD method for assessing neuritic plaques[86] and a topographic staging method for neurofibrillary degeneration comparable to that proposed by Braak and Braak.[89]
- Lewy body type pathology should be assigned according to the previous guidelines in the original Consensus report.[1] Semiquantitative grading of Lewy body severity should be adopted rather than counting LB in various brain regions.
- The following scoring system for LB is recommended (figure):

  0 = None

  1 = Mild (sparse LBs or LNs)

  2 = Moderate (more than one LB in a low power field and sparse LNs)

  3 = Severe (four or more LBs and scattered LNs in a low power field)

  4 = Very severe (numerous LBs and numerous LNs)

While brainstem nuclei are affected in virtually every case of LB disease, the severity of brainstem pathology is highly variable. Similarly, there is a range of severity of involvement in the various limbic and neocortical regions; thus, for most areas a range of severity is acceptable. The pattern of regional involvement is more important than total LB count. Table 2 presents a scheme for assigning LB disease type by assessing the regional pattern of Lewy-related pathology using CERAD-like scoring for LB.

Table 3 shows criteria for allocating a probability that neuropathologic findings will be associated with a DLB clinical syndrome taking account of both AD and LB type pathology.

As in the NIA-Reagan criteria, SP types should be subclassified as diffuse and neuritic but for diagnostic purposes, only neuritic plaques should be considered.

Specification for the assessment of vascular pathology in DLB was made in the original consensus statement document and in the absence of further significant research findings it is recommended to continue using this approach.

Neuropathologic research strategies. A scheme to stage Lewy-related pathology in the brain has been proposed for PD.[90] The validity of staging and its relevance to DLB remains to be determined by its application to brains of prospectively studied individuals with a range of cognitive and extrapyramidal dysfunction. Similarly, while considerable research has been reported on Lewy-related pathology in the amygdala and periamygdaloid cortex using immunostaining for alpha-synuclein, additional studies are warranted in prospectively studied cohorts in order to understand possible clinical correlates of this pathology in DLB as well as in AD, where this may be the only brain region with alpha-synuclein pathology.[91,92] Critical to this issue is the clinical significance, if any, of this pattern of alpha-synuclein pathology. As such, the presence or absence of LB in the amygdala should be documented in all cases of dementia reaching neuropathologic autopsy. Determining the presence of alpha-synuclein pathology in the amygdala in other dementias is a related research objective.

It is clear from several case studies that familial cases of DLB occur[93,94] and that LBs are commonly

Table 3 Assessment of the likelihood that the pathologic findings are associated with a DLB clinical syndrome

	Alzheimer type pathology		
	NIA-Reagan Low (Braak stage 0–II)	NIA-Reagan Intermediate (Braak stage III–IV)	NIA-Reagan High (Braak stage V–VI)
Lewy body type pathology			
Brainstem-predominant	Low	Low	Low
Limbic (transitional)	High	Intermediate	Low
Diffuse neocortical	High	High	Intermediate

DLB = dementia with Lewy bodies; NIA = National Institute on Aging.

seen in familial cases of AD.[95] There are recent reports that triplication of the alpha-synuclein gene (SNCA) can cause DLB, PD, and PDD whereas gene duplication is associated only with motor PD, suggesting a gene dose effect.[96] However, SCNA multiplication is not found in most patients with LB disease.[97] Continued clinical, pathologic, and genetic evaluation of familial cases of DLB and AD is therefore an important and potential highly informative area for continued research.

Clinical management. Patient management in DLB is complex and includes early detection, investigation, diagnosis, and treatment of cognitive impairment; assessment and management of neuropsychiatric and behavioral symptoms; treatment of the movement disorder; and monitoring and management of autonomic dysfunction and sleep disorders.[98] The evidence base for making recommendations about the management of DLB is limited and what follows is based upon consensus opinion of clinicians experienced in treating DLB.

Nonpharmacologic interventions. Nonpharmacologic interventions have the potential to ameliorate many of the symptoms and functional impairments associated with DLB, but none has yet been systematically evaluated. Cognitive dysfunction and associated symptoms such as VH can for example be exacerbated by low levels of arousal and attention and strategies to increase these by social interaction and environmental novelty may reduce their presence and impact.

Pharmacologic treatments. Motor parkinsonism. Levodopa can be used for the motor disorder of both DLB and PDD.[18,19] Medication should generally be introduced at low doses and increased slowly to the minimum required to minimize disability without exacerbating psychiatric symptoms. Anticholinergics should be avoided.

Neuropsychiatric symptoms. Visual hallucinations are the most commonly experienced psychiatric symptom and are often accompanied by delusions, anxiety, and behavioral disturbance. When pharmacologic intervention is required the options include cholinesterase inhibitors (CHEIs) or atypical antipsychotic medications. Open label studies have demonstrated the effectiveness of all three generally available CHEIs in DLB and PDD but placebo controlled trial data are only available to date for rivastigmine.[27,28] The reported reduction in symptom frequency and intensity of VH appears to be mediated at least in part by improved attentional function and the presence of VH is associated with greater cognitive improvement.[47] Side effects of hypersalivation, lacrimation, and urinary frequency may occur, in addition to the usual gastrointestinal symptoms, and a dose dependent exacerbation of extrapyramidal motor features may occur in a minority. If CHEIs are ineffective or if more acute symptom control of behavior is required, it may be difficult to avoid a cautious trial of an atypical antipsychotic. The clinician should warn both the carer and patient of the possibility of a severe sensitivity reaction.[26] Typical antipsychotics should be avoided.[55] Novel atypicals with potentially more favorable pharmacologic properties, such as quetiapine, clozapine, and aripiprazole, may have theoretical advantages over traditional agents in LB disease[99-101] but controlled clinical trial data are needed.

Depression is common in both DLB and PDD and there have been no systematic studies of its management. At the present time SSRI and SNRIs are probably preferred pharmacologic treatment. Tricyclic antidepressants and those with anticholinergic properties should generally be avoided. Apathy is also common and may improve with CHEIs.[27] Sleep disorders are frequently seen in LB disease and may be an early feature. RBD can be treated with clonazepam 0.25 mg at bedtime, melatonin 3 mg at bedtime, or quetiapine 12.5 mg at bedtime and titrated slowly monitoring for both efficacy and side effects.[53] CHEIs may be helpful for disturbed sleep.[102]

Cognitive symptoms. CHEIs may be of benefit for the fluctuating cognitive impairments with impact on global function and activities of daily living.[103] The effect size in DLB is reported as being generally larger than seen with the same drugs when used in AD.[104] Only limited data on long-term effects are available[105] and there are none about possible disease-modifying effects.

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Anti-Parkinsonian agents have anti-amyloidogenic activity for Alzheimer's β-amyloid fibrils in vitro

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Abstract

Inhibition of the accumulation of amyloid β-peptide (Aβ) and the formation of β-amyloid fibrils (fAβ) from Aβ, as well as the destabilization of preformed fAβ in the central nervous system would be attractive therapeutic targets for the treatment of Alzheimer's disease (AD). Many studies have demonstrated that oxidative damage plays a central role in AD pathogenesis, as well as Parkinson disease (PD). Among the antioxidant strategies proposed, increasing evidence points to the possibility of achieving neuroprotection by dopamine agonists, as well as monoamine oxidase B (MAO-B) inhibitors. Actually, the beneficial effect of selegiline, a MAO-B inhibitor, in AD has been noted in several clinical studies. On the reverse, antimuscarinic agents have been reported to accelerate β-amyloidosis and senile plaque formation in PD. Using fluorescence spectroscopic analysis with thioflavin T and electron microscopic studies, we examined the effects of anti-Parkinsonian agents, dopamine, levodopa, pergolide, bromocriptine, selegiline, and trihexyphenidyl on the formation, extension, and destabilization of fAβ(1-40) and fAβ(1-42) at pH 7.5 at 37 °C in vitro. The anti-Parkinsonian agents other than trihexyphenidyl dose-dependently inhibited fAβ formation from Aβ(1-40) and Aβ(1-42), as well as their extension. Moreover, these agents dose-dependently destabilized preformed fAβs. The overall activity of the molecules examined was in the order of: dopamine > selegiline > levodopa = pergolide > bromocriptine. Although the exact mechanism of the anti-amyloidogenic activity of these agents is unclear, these and other structurally related compounds could be key molecules for the development of therapeutics for AD and other conformational diseases.

Keywords: Alzheimer's disease; Anti-Parkinsonian agents; β-Amyloid fibrils; Thioflavin T; Electron microscopy

1. Introduction

Alzheimer's disease (AD) is characterized by the abundance of intraneuronal neurofibrillary tangles and the extracellular deposition of the amyloid β-peptide (Aβ) as amyloid plaques and vascular amyloid (Selkoe, 2001). Despite recent progress in the symptomatic therapy with cholinergic drugs, an effective therapeutic approach that interferes directly with the neurodegenerative process in AD, especially the accumulation of Aβ in the CNS is eagerly awaited. Recently, many researchers favor therapeutic approaches that target the formation, deposition and clearance of Aβ from nerve tissue. Experimental therapies and clinical trials using vaccination (Schenk et al., 1999) and non-steroidal anti-inflammatory drugs (McGeer and McGeer, 1999) have been reported.

Many studies have revealed that oxidative damage plays a central role in AD pathogenesis (Pratico and Delanty, 2000). Many antioxidant compounds, such as Vitamin E (Subramaniam et al., 1998), nordihydroguaiaretic acid (NDGA) (Goodman et al., 1994), and nicotine (Kihara et al., 1997) have been demonstrated to protect the brain from in vitro Aβ toxicity, and clinical trials to test the ability of high dose Vitamin E to slow AD progression have been carried out (Sano et al., 1997; Morris et al., 2002). Recently, we showed that the antioxidants such as the Indian spice curcumin (Cur), its analog rosmarinic acid

Abbreviations: Aβ, amyloid β-peptide; AD, Alzheimer's disease; Bro, bromocriptine; Cat, catechin; Cur, curcumin; DMSO, dimethyl sulfoxide; EC_{50}, effective concentrations at 50% value; epi-Cat, epicatechin; fAβ, β-amyloid fibrils; Kmp, kaempferol; L-Dopa, levodopa; MAO-B, monoamine oxidase B; Mor, morin; Myr, myricetin; NDGA, nordihydroguaiaretic acid; PD, Parkinson's disease; Per, pergolide; Qur, quercetin; RA, rosmarinic acid; RIF, rifampicin; Sel, selegiline hydrochloride; TA, tannic acid; TC, tetracycline; ThT, thioflavin T; Tri, trihexyphenidyl hydrochloride

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(RA), and wine-related polyphenols dose-dependently inhibit formation and extension of fAβ(1-40) and fAβ(1-42), as well as destabilizing preformed fAβs in vitro (Ono et al., 2003, 2004a).

On the other hand, another major neurodegenerative disease, Parkinson's disease (PD) is a progressive neurodegenerative disease that is characterized by impairment of motor function such as bradykinesia, rest tremor, rigidity, gait abnormalities and postural instability (Chung et al., 2003). Pathologically, PD is characterized by the presence of Lewy bodies and a selective loss of dopaminergic neurons in the substantia nigra pars compacta (Chung et al., 2003). As in AD, it is widely accepted that oxidative stress underlies the selective vulnerability of dopaminergic neurons observed in PD (Chung et al., 2003). In general, symptomatic treatment of PD is partially successful with levodopa which is still regarded as the standard and most effective form of therapy, although the long-term use of this agent is complicated by loss of efficacy and the development of a variety of motor complications such as dyskinesias (Gómez-Vargas et al., 1998; Chung et al., 2003). Based on the evidence for an oxidative component in PD, and in view of the complications associated with levodopa therapy, an alternative approach to the treatment of this disorder would be the use of neuroprotective or antioxidant therapy to prevent or slow down the degeneration of these neurons (Gómez-Vargas et al., 1998). Among the antioxidant strategies proposed, increasing evidence points to the possibility of achieving neuroprotection by administration of dopamine agonists, such as pergolide or bromocriptine, as well as monoamine oxidase B (MAO-B) inhibitors, such as selegiline (L-deprenyl, phenylisopropyl-*N*-methylpropylamine) (Wu et al., 1993; Ogawa et al., 1994; Gómez-Vargas et al., 1998). Actually, the beneficial effect of selegiline in AD has been noted in several clinical studies (Mangoni et al., 1991; Sano et al., 1997). Anti-muscarinic agents, such as trihexyphenidyl, have been reported to increase the frequency of dementia (McKeith, 2000) and accelerate β-amyloidosis and senile plaque formation in the aging brain in subjects with PD (Perry et al., 2003). Thus, we hypothesized that dopamine agonists and MAO-B inhibitors inhibit fAβ formation, while anti-muscarinic agents promote fAβ formation.

Using a nucleation-dependent polymerization model to explain the mechanism of the formation of Alzheimer's β-amyloid fibrils (fAβ) in vitro (Jarrett and Lansbury, 1993; Naiki et al., 1997), we examined the effects of anti-Parkinsonian agents, dopamine hydrochloride, levodopa (L-3,4-dihydroxyphenylalanine) (L-Dopa), pergolide (Per), bromocriptine (Bro), selegiline hydrochloride (Sel), and trihexyphenidyl hydrochloride (Tri) to inhibit the formation, extension of fAβ(1-40) and fAβ(1-42), as well as to destabilize fAβs at pH 7.5 at 37 °C in vitro, using fluorescence spectroscopy with ThT and electron microscopy.

2. Experimental procedures

2.1. Preparation of Aβ and fAβ solutions

Aβ(1-40) (a trifluoroacetate salt, lot number 530108, Peptide Institute Inc., Osaka, Japan) and Aβ(1-42) (a trifluoroacetate salt, lot number 521205, Peptide Institute) were dissolved by brief vortexing in a 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 Aβ(1-40) and Aβ(1-42) solutions). fAβ(1-40) and fAβ(1-42) were formed from the fresh Aβ(1-40) and Aβ(1-42) solutions, respectively, sonicated, and stored at 4 °C as described elsewhere (Hasegawa et al., 1999).

Fresh, non-aggregated fAβ(1-40) and fAβ(1-42) were obtained by extending sonicated fAβ(1-40) or fAβ(1-42) with fresh Aβ(1-40) or Aβ(1-42) solutions, respectively, just before the destabilization reaction (Ono et al., 2002b). The reaction mixture was 600 μL and contained 10 μg/mL (2.3 μM) fAβ(1-40) or fAβ(1-42), 50 μM Aβ(1-40) or Aβ(1-42), 50 mM phosphate buffer, pH 7.5, and 100 mM NaCl. Measurement of the fluorescence of ThT showed that the extension reaction proceeded to equilibrium after incubation at 37 °C for 3–6 h under non-agitated conditions.

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

The fluorescence spectroscopic study was performed on a Hitachi F-2500 fluorescence spectrophotometer as described elsewhere (Naiki and Nakakuki, 1996). Optimum fluorescence measurements of fAβ(1-40) and fAβ(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. Electron microscopic and polarized light microscopic studies of the reaction mixtures were performed as described elsewhere (Hasegawa et al., 1999).

2.3. Polymerization assay

Polymerization of Aβ with or without fAβ added as seeds was assayed as described elsewhere (Naiki et al., 1998). Briefly, the reaction mixture contained 50 μM Aβ(1-40), or 25 or 50 μM Aβ(1-42), 0 or 10 μg/mL fAβ(1-40) or fAβ(1-42), 0–100 μM NDGA, RIF or anti-Parkinsonian agents (dopamine, L-Dopa, Per, Bro, Sel, Tri), 1% dimethyl sulfoxide (DMSO), 50 mM phosphate buffer, pH 7.5, and 100 mM NaCl. NDGA, RIF, dopamine, L-Dopa, Bro, Per (Sigma Chemical Co., St. Louis, MO), and Sel (Fujimoto Pharma., Osaka, Japan) were first dissolved in DMSO at concentrations of 1, 10, 100 μM, 1 and 5 mM, then added to the reaction mixture to make the final concentrations 0.01, 0.1, 1, 10 and 50 μM, respectively. Tri was first dissolved in DMSO at concentrations of 5 and 10 mM then added to the reaction mixture to make the final concentrations 50 and 100 μM, respectively.

Thirty microliters aliquots of the mixture were put into oil-free PCR tubes (size; 0.5 mL, code number; 9046, Takara Shuzo Co. Ltd., Otsu, Japan). These tubes were then put into a DNA thermal cycler (PJ480, Perkin-Elmer Cetus, Emeryville, CA). Starting at 4 °C, the plate temperature was elevated at maximal speed, 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 tubes were not agitated during the reaction. Five microliters aliquots from each tube in triplicate were subjected to fluorescence spectroscopy and the mean of the three measurements was determined. In the ThT solution, the solutions of NDGA, RIF, dopamine, L-Dopa, Per, Bro, Sel and Tri examined in this study were diluted up to 1/200 of the concentration in the reaction mixture. We confirmed that these compounds did not quench ThT fluorescence at the diluted concentration (data not shown).

2.4. Measurement of fibril-destabilizing activity

Destabilization of fAβ was assayed as described elsewhere (Ono et al., 2002b). Briefly, the reaction mixture contained 25 μM fresh fAβ(1-40) or fAβ(1-42), 0–100 μM NDGA, RIF, or anti-Parkinsonian agents, 1% DMSO, 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 1% (wt./vol) polyvinyl alcohol (Wako Pure Chemical Industries Ltd., Osaka, Japan) to avoid the aggregation of fAβ and the adsorption of fAβ onto the inner wall of the reaction tube during the reaction. NDGA, RIF, dopamine, L-Dopa, Per, Bro and Sel dissolved in DMSO at concentrations of 1, 10, 100 μM, 1 and 5 mM, were added to the reaction mixture to make the final concentrations 0.01, 0.1, 1, 10 and 50 μM, respectively. Tri was first dissolved in DMSO at concentrations of 5

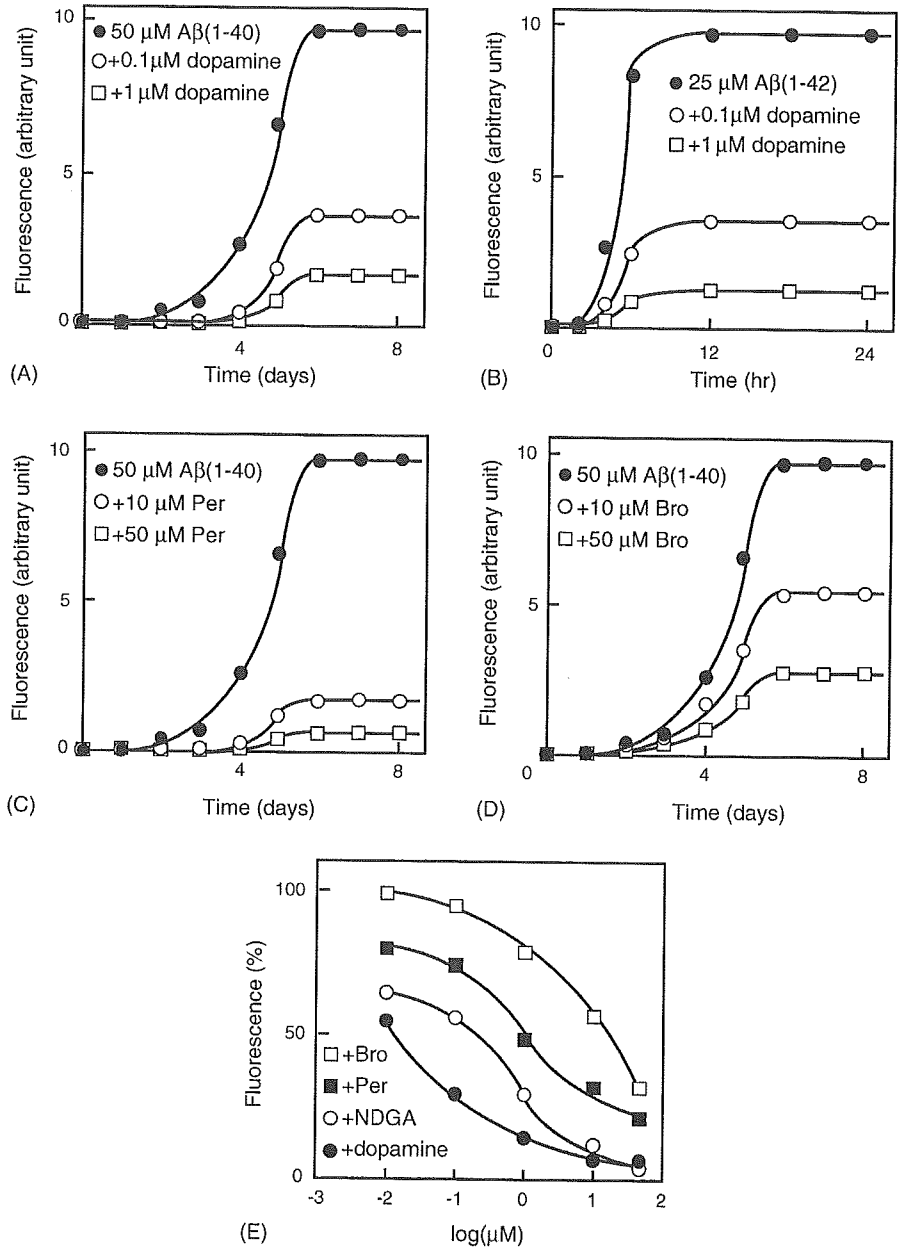

Fig. 1. (A–D) Effects of dopamine (A and B), Per (C), and Bro (D) on the kinetics of formation of fAβ(1-40) (A, C, D) and fAβ(1-42) (B) from fresh Aβ(1-40) and Aβ(1-42), respectively. The reaction mixtures containing 50 μM Aβ(1-40) (A, C and D) or 25 μM Aβ(1-42) (B), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 μM (●), 0.1 μM (○) or 1 μM (□) of dopamine (A and B), or 0 μM (●), 10 μM (○) or 50 μM (□) of Per (C) or Bro (D), were incubated at 37 °C for the indicated times. Each figure shows a representative pattern of three independent experiments. (E) Dose-dependent inhibition of fAβ(1-40) formation from fresh Aβ(1-40). The reaction mixtures containing 50 μM Aβ(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0, 0.01, 0.1, 1, 10 or 50 μM dopamine (●), NDGA (○), Per (■) or Bro (□) were incubated at 37 °C for 7 days. Each point represents the mean of three independent experiments. At all points, standard errors were within symbols. The average without compounds was regarded as 100%.

and 10 mM then added to the reaction mixture to make the final concentrations 50 and 100 μM, respectively.

After being mixed by pipetting, triplicate 5 μL aliquots of the reaction mixture were subjected to fluorescence spectroscopy and 30 μL aliquots were put into PCR tubes. The reaction tubes were then put into a DNA thermal cycler. Starting at 4 °C, the plate temperature was elevated at maximal speed to 37 °C. Incubation times ranged between 0 and 6 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. Five microliters aliquots from each tube in triplicate were subjected to fluorescence spectroscopy and the mean of the three measurements was determined. When fAβ were incubated with 0–50 μM of these compounds at either 4 °C or 37 °C for 1 min, then subjected to ThT assay, ThT fluorescence did not change significantly, indicating that these compounds did not compete with ThT for fAβ (data not shown).

2.5. Other analytical procedures

Protein concentrations of the supernatants of the reaction mixtures after centrifugation were determined by the method of Bradford (1976) with a protein

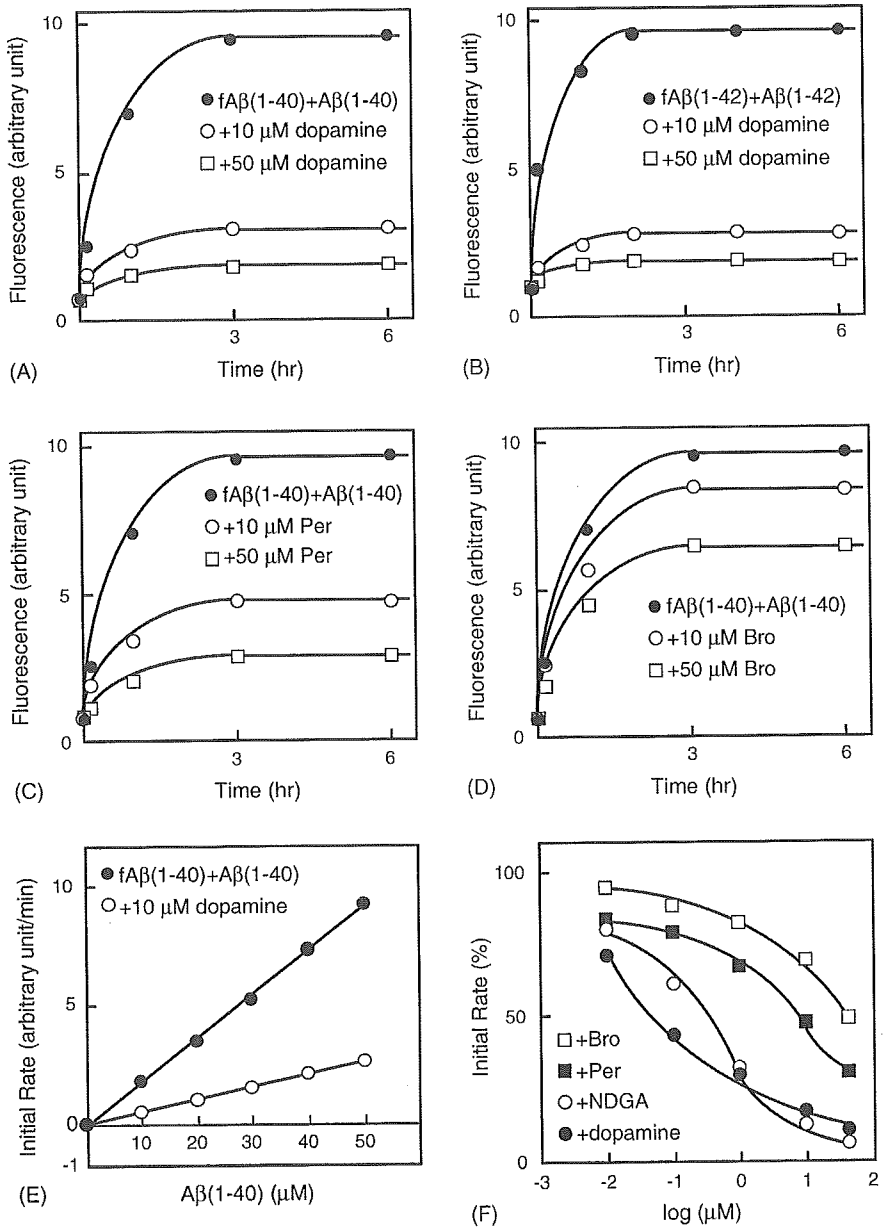

Fig. 2. (A–D) Effects of dopamine (A and B), Per (C) and Bro (D) on the kinetics of fAβ(1-40) (A, C and D) and fAβ(1-42) (B) extension. The reaction mixtures containing 10 μg/mL (2.3 μM) sonicated fAβ(1-40) (A, C, D) or fAβ(1-42) (B), 50 μM Aβ(1-40) (A, C and D) or Aβ(1-42) (B), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 μM (●), 10 μM (○) or 50 μM (□) of dopamine (A and B), Per (C), or Bro (D), were incubated at 37 °C for the indicated times. Each figure shows a representative pattern of three independent experiments. (E) Effect of Aβ(1-40) concentration on the initial rate of fAβ(1-40) extension in the presence (○) or absence (●) of dopamine. The reaction mixtures containing 10 μg/mL (2.3 μM) sonicated fAβ(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, 0 (●) or 10 μM (○) dopamine, and 0, 10, 20, 30, 40 or 50 μM Aβ(1-40), were incubated at 37 °C for 1 h. Each point represents the mean of three independent experiments. At all points, standard errors were within symbols. Linear least-square fit was performed for each straight line (R^2 = 0.998 and 0.999 for (●) and (○), respectively). (F) Dose-dependent inhibition of fAβ(1-40) extension. The reaction mixtures containing 10 μg/mL (2.3 μM) sonicated fAβ(1-40), 50 μM Aβ(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0, 0.01, 0.1, 1, 10 or 50 μM dopamine (●), NDGA (○), Per (■) or Bro (□) were incubated at 37 °C for 1 h. Each point represents the mean of three independent experiments. At all points, standard errors were within symbols. The average without compounds was regarded as 100%.

assay kit (Bio-Rad Laboratories Inc., Hercules, CA). The Aβ(1-40) solution quantified by amino acid analysis was used as the standard. The statistical significance of the data was analyzed by the linear least squares fit. The effective concentration (EC_{50}) was defined as the concentration of NDGA, RIF, dopamine, L-Dopa, Per, Bro or Sel to inhibit the formation or extension of fAβs to 50% of the control value, or the concentration to destabilize fAβs to 50% of the control value. EC_{50} was calculated by the sigmoidal curve fitting of the data as shown in Figs. 1, 2 and 4, using Igor Pro ver.4 (WaveMetrics Inc., Lake Oswego, OR, USA). Correlation analysis was performed according to Pearson's correlation test. Pearson's correlation coefficients were calculated to determine the relationship between the EC_{50} to inhibit the formation of fAβs and the EC_{50} to destabilize fAβs.

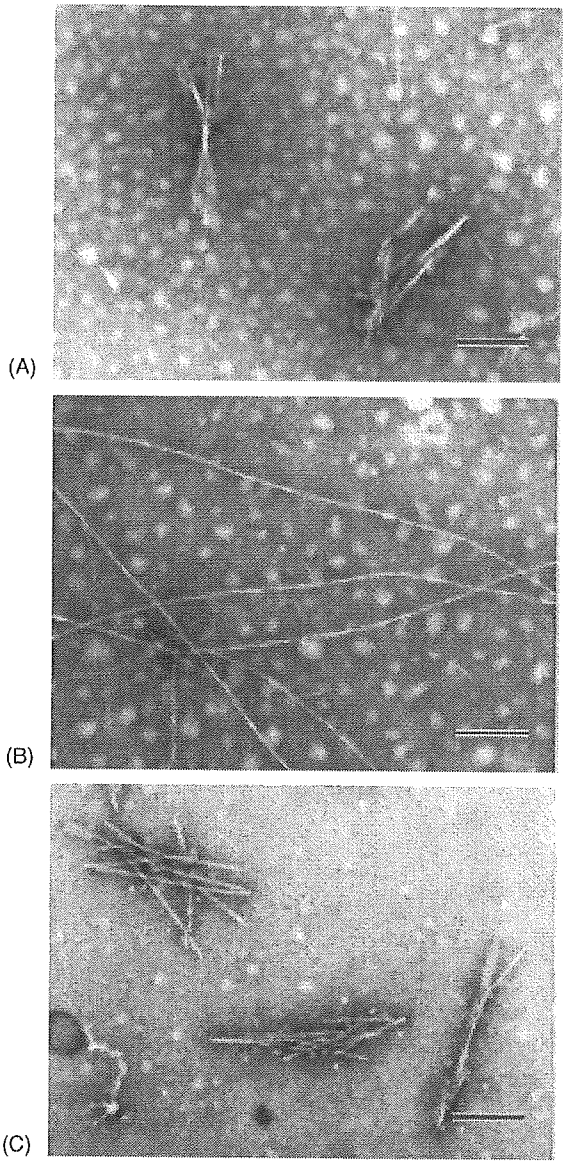

Fig. 3. Electron micrographs of extended fAβ(1-40). The reaction mixtures containing 10 μg/mL (2.3 μM) fAβ(1-40), 50 μM Aβ(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (B) or 50 μM dopamine (A and C), were incubated at 37 °C for 0 (A) or 6 h (B and C). Scale bars indicate a length of 250 nm.

3. Results

3.1. Effect of anti-Parkinsonian agents on the kinetics of fAβ formation

As shown in Fig. 1A–D, when fresh Aβ(1-40) or Aβ(1-42) was incubated at 37 °C, the fluorescence of ThT followed a characteristic sigmoidal curve. This curve is consistent with a nucleation-dependent polymerization model (Jarrett and Lansbury, 1993; Naiki et al., 1997). fAβ(1-40) and fAβ(1-42) stained with Congo red showed typical orange–green birefringence under polarized light (data not shown). When Aβ(1-40) was incubated with 0.1 and 1 μM dopamine, the final equilibrium level decreased dose-dependently (Fig. 1A). A similar effect of dopamine was observed with Aβ(1-42) (Fig. 1B). When Aβ(1-40) was incubated with 10 and 50 μM L-Dopa, Per, Bro or Sel, the final equilibrium level decreased dose-dependently (Fig. 1C and D, data not shown). A similar effect of L-Dopa, Per, Bro and Sel was observed with Aβ(1-42) (data not shown). Tri at 50 and 100 μM had no inhibitory effect on the polymerization (data not shown).

As shown in Fig. 2A–D, when fresh Aβ(1-40) or Aβ(1-42) was incubated with the seeds fAβ(1-40) or fAβ(1-42), respectively, at 37 °C, the fluorescence increased hyperbolically without a lag phase and proceeded to equilibrium much more rapidly than without the seeds fAβ(1-40) or fAβ(1-42) (compare Figs. 1 and 2). This curve is consistent with a first-order kinetic model (Naiki and Nakakuki, 1996). When Aβ(1-40) and fAβ(1-40) were incubated with dopamine, L-Dopa, Per, Bro or Sel, the final equilibrium level decreased (Fig. 2A, C and D, data not shown). A similar effect of these agents was observed on the extension of fAβ(1-42) (Fig. 2B, data not shown). Tri at 50 and 100 μM had no inhibitory effect on the extension of either fAβ(1-40) or fAβ(1-42) (data not shown). At a constant fAβ(1-40) concentration (10 μg/mL), a perfect linearity was observed between the Aβ(1-40) concentration and the initial rate of fAβ(1-40) extension both in the presence and absence of dopamine (Fig. 2E). This linearity is again consistent with a first-order kinetic model and indicates that at each Aβ(1-40) concentration, the net rate of fAβ(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 dopamine, the slope of the straight line decreased to about 3/10. The mechanism of the antiamyloidogenic effect of dopamine will be discussed later.

When fresh Aβ(1-40) was incubated with fAβ(1-40) at 37 °C, clear fibril extension was observed electron-microscopically (Fig. 3B). However, 50 μM dopamine completely inhibited the extension of sonicated fAβ(1-40) (Fig. 3A and C). Dopamine inhibited the extension of fAβ(1-42) (data not shown). Similarly, L-Dopa, Per, Bro and Sel also inhibited the extension of fAβ(1-40) and fAβ(1-42) (data not shown).

3.2. Fibril-destabilizing assay

As shown in Fig. 4A–D, the fluorescence of ThT was almost unchanged during the incubation of fresh fAβ(1-40) or fAβ(1-42) at 37 °C without additional molecules. On the other hand, the ThT fluorescence decreased immediately after addition of the anti-Parkinsonian agents other than Tri to the reaction mixture. After incubation of 25 μM fresh fAβ(1-40) with 50 μM dopamine for 1 h, many short, sheared fibrils were observed (Fig. 5B). At 6 h, the number of fibrils was reduced markedly, and small amorphous aggregates were occasionally observed (Fig. 5C). Similar morphology was observed when 25 μM fresh fAβ(1-42) were incubated with 50 μM dopamine (data not shown). L-Dopa, Per, Bro and Sel also destabilized preformed fAβ(1-40) and fAβ(1-42) similarly (data not shown). Tri at 50 and 100 μM had no destabilizing effect for either preformed fAβ(1-40) or fAβ(1-42) (data not shown).

After incubation with 50 μM dopamine, L-Dopa, Per, Bro or Sel for 6h, fAβ(1-40) and fAβ(1-42) were stained with Congo

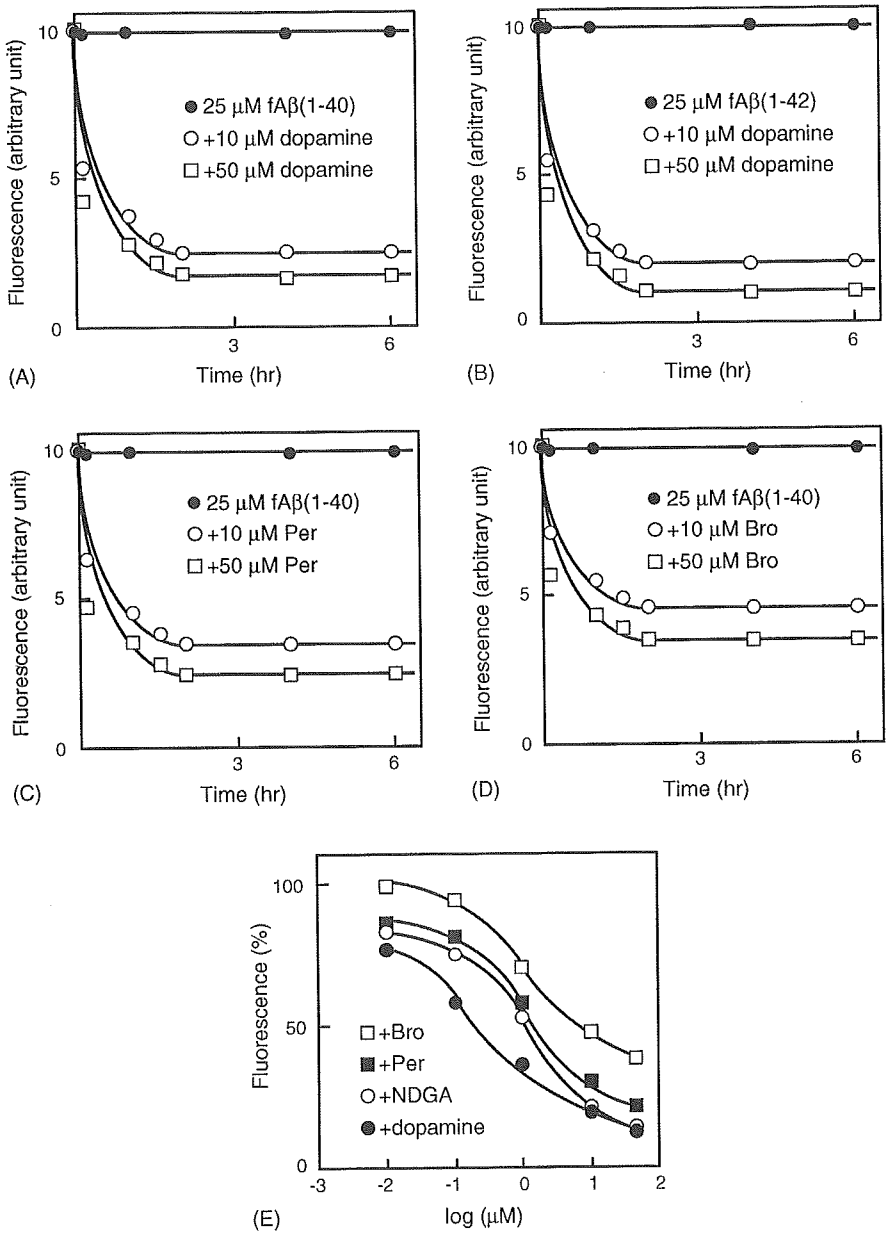

Fig. 4. (A–D) Effects of dopamine (A and B), Per (C) and Bro (D) on the kinetics of destabilization of fAβ(1-40) (A, C and D) and fAβ(1-42) (B). The reaction mixtures containing 25 μM fAβ(1-40) (A, C and D) or fAβ(1-42) (B), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 μM (●), 10 μM (○) or 50 μM (□) of dopamine (A and B), Per (C) or Bro (D), were incubated at 37 °C for the indicated times. Each figure shows a representative pattern of three independent experiments. (E) Dose-dependent destabilization of fAβ(1-40). The reaction mixtures containing 25 μM fAβ(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl and 0, 0.01, 0.1, 1, 10 or 50 μM dopamine (●), NDGA (○), Per (■) or Bro (□) were incubated at 37 °C for 6 h. Each point represents the mean of three independent experiments. At all points, standard errors were within symbols. The average without compounds was regarded as 100%.

red much more weakly than fresh fAβ(1-40) and fAβ(1-42). However, they all showed orange–green birefringence under polarized light. This means that a significant amount of intact fAβ(1-40) and fAβ(1-42) still remains in the mixture after the reaction. When the protein concentration of the supernatant after centrifugation at 4 °C for 2 h at 1.6×10^4 *g* was measured by the Bradford assay, no proteins were detected in the supernatant in any case. This implies that although these agents could destabilize fAβ(1-40) and fAβ(1-42) to visible aggregates (Fig. 5C), they could not depolymerize fAβ(1-40) and fAβ(1-42) to monomers or oligomers of Aβ(1-40) and Aβ(1-42). When fresh 50 μM Aβ(1-40) or Aβ(1-42) was incubated with 10 μg/mL of the pellet, no increase in the fluorescence was observed for 6 h. This implies that destabilized fAβ(1-40) and fAβ(1-42) could not function as seeds.

3.3. Comparison of the activity of anti-Parkinsonian agents

As shown in Figs. 1E, 2F and 4E, NDGA, RIF and the anti-Parkinsonian agents other than Tri dose-dependently inhibited the formation and extension of fAβs, and dose-dependently destabilized preformed fAβs. We calculated EC_{50}, i.e., the concentrations of NDGA, RIF or other agents to inhibit the formation or extension of fAβs to 50% of the control value, or