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Scientific board

THE SECOND INTERNATIONAL CONFERENCE OF BRAIN AGING

- The South-East European Society on Brain Aging

INTERNATIONAL TRAINING COURSE OF BRAIN AGING

October 13 - 16, 2005, Seifenfabrik Veranstaltungszentrum - Graz - Austria

Dear Colleagues and Friends,

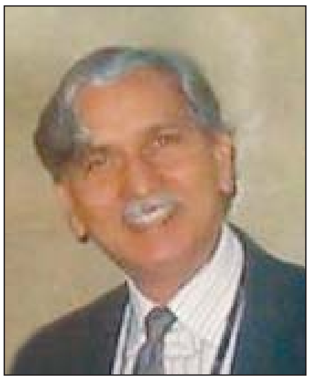
On behalf of the "Ana Aslan" International Academy of Aging and The South-East European Society on Brain Aging, it is our great pleasure to invite you to attend the Second International Conference of Brain Aging, followed by an Intensive Post Graduate Training in Dementias, which will be held on October 13-16, 2005 in Graz, Austria.

Prof. Bengt Winblad, MD, PhD

Ass. Prof. Luiza Spiru, MD, PhD

The conference program will brought up to date discovers in the field of brain aging, and papers on different aspects of dementias and memory impairment diseases will be presented by the **Editorial and Scientific Board of the Brain Aging International Journal**, as well by **well known scientists in this field, from the South-East European Countries.**

The goal of this training is to provide scientists from European, especially the Eastern European Countries, skills in the **early diagnosis and treatment of Alzheimer's disease and other dementias disorders**, and working with dementia cases.



Prof. Khalid Iqbal, PhD

We are looking forward to encourage the participants to take the advantages of such a high level international meeting on aging, which will gather for the first time geriatricians, neurologists, psychiatrists, general practitioners, special care researchers, persons working in the field of dementia care, testing supervisors and researchers from European Countries.

Bengt Winblad, M.D., Ph.D	Luiza Spiru, M.D., Ph.D.	Khalid Iqbal, Ph.D.

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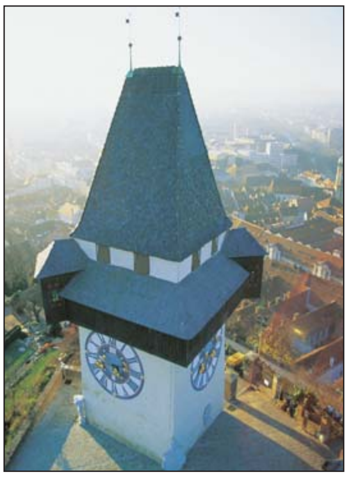
Attendance fees

Type of participant	Early registration until 31st of July 2005	Late registration 1st of August 2005 to Brain Aging Conference
Full delegate	€ 300	€ 350
Presenter of oral presentation or poster (after successful review)	€ 200	€ 250
Student, young scientists (age limit 35 years, letter of support)	€ 100	€ 150

Topics



- ♦ Brain Aging and Dementia ♦ Therapeutically Strategies in AD
- ♦ Biomarkers Imaging Neuropsychological Inventory in AD
- ♦ Non-Alzheimer Dementias ♦ MCI - Definition to Treatment
- ♦ Depression in the Elderly versus Depression in Dementia
- ♦ Ethical issues in Dementias ♦ Basic Mechanisms Involved in Brain Aging
- ♦ Neuropathology Disorders ♦ Plaque Pathology ♦ Hereditary Forms of Dementia
- ♦ Clinical Trials - Ethical considerations involved in Clinical Trials
- ♦ Guide Lines for Diagnosis in Dementias ♦ Memory Training ♦ Genetics in AD
- ♦ Nutritional Brain Aging ♦ High Blood Pressure and Consequences for Brain Aging
- ♦ Lipid Lowering Compounds and Brain Aging
- ♦ Harmonization of Treatment Strategies of Age Related Brain Diseases in S-E Europe

International Training Course of Brain Aging

- ♦ Epidemiological Aspects of Dementias Diseases
- ♦ Genetics Aspects of AD Neuropsychological Assessment
- ♦ Neuropathology, Neuroimaging in Dementia
- ♦ Neuropsychological
- ♦ Differential Diagnosis of AD
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Discriminators of, and Treatment Strategies for, the Dementias of Parkinsonism

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Abstract

Dementia is an important cause of morbidity and mortality in Parkinson's disease. Patients with Parkinson's disease (Pd) are six times more likely to develop dementia than the general population, with an incidence rate of around 95 per 1000 person years. The risk factors for development of dementia in Pd include older age, longer disease duration, lower mini mental state examination score and more severe parkinsonism. Parkinson's plus dementia can be discriminated from dementia with Lewy bodies (DLB) pathologically by the predominance of Lewy bodies in the cortex rather than the brain stem. However effective clinical discriminators between DLB and Pd plus dementia are shorter disease course and early cognitive and psychiatric symptoms in the DLB group. Treatments of psychotic symptoms in Pd dementia include atypical antipsychotics and acetylcholinesterase inhibitors (AChEI). Of the atypical antipsychotics, clozapine and quetiapine appear to produce the least deterioration in motor function. Treatment strategies with AChEI are in evolution and appear promising but further trials are needed.

Keywords: *Dementia, Lewy bodies, Parkinsonism, Parkinson's disease*

Individuals with Parkinson's disease (Pd), or who suffer parkinsonism (hereafter referred to as PS), may manifest a variety of psychiatric conditions. These include depression, anxiety, delirium, behavioural problems, cognitive impairment, and dementia. Whilst there has been much research into the neuropathology, treatment and risk factors for dementia in PS, little attempt has been made to draw together a structured treatment option. We have reviewed the state of knowledge of the dementias of PS, and this has allowed us to suggest logical treatment strategies, which are discussed below.

Clinical context

The consequences of developing cognitive deficit in PS are severe. In addition to the obvious decline in function, demented Pd patients have an increased mortality compared with non-demented Pd patients. The reasons for this are not entirely clear. In their prospective study of mortality in elderly individuals with or without Pd, Louis et al found that the relative risk for mortality in those with Pd and dementia was 4.9 (95% CI 3.4-7.1, P<0.001) when compared to a group of elderly controls matched for age, sex and smoking history. The relative risk for mortality was 2.7 (1.7-4.4, P<0.001) in patients with Pd alone[2]. A high extrapyramidal symptoms score (EPS), a measure of severity of the motor manifestations of Pd, was the most important indicator of increased mortality. The mean baseline EPS was higher in those with Pd and dementia, inferring a higher EPS rather than the presence of dementia may have contributed to greater mortality in patients with both Pd and dementia. However dementia increased mortality even in those with lower EPS. Pneumonia and cerebrovascular causes of death were commoner in Pd patients with dementia as compared to those with

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Pd alone or controls. Dementia places a severe financial burden on carers, health and social services. Pd sufferers with dementia are more likely to be placed in a nursing home, often due to carer distress[1]. It is therefore important to determine the risk factors and the underlying mechanisms that contribute to the development of dementia in PS sufferers. To do this, the incidence of dementia in these patients requires quantification.

Risk of developing dementia in Parkinson's disease

It is difficult to determine dementia incidence accurately in PS with dementia when compared to Pd without dementia, owing to the higher mortality observed in the former group. Cross sectional prevalence studies may well underestimate the risk of developing dementia in these groups. One UK based study gives an incidence of dementia in Pd of 42.6 per 1000 person-years (the number of person years being the time in years between start of illness or baseline and follow up for each individual)[7]. However the subjects in this study were younger (median 73.2 years, range 53-81years) and the disease duration shorter, suggesting that they may not have been a representative sample of Pd sufferers. It is notable that during follow up none of the controls developed dementia. Thus it was not possible to provide a relative risk for the development of dementia in Pd. Marder et al (1995) derived an incidence of 112.5 per 1000 person years during a two year follow up period of 140 elderly non-demented patients with Pd[8]. But an unusually high incidence of dementia in the control group (80/1000 person years) contributed to a relative risk of dementia in Pd subjects, as compared to the controls, of 1.7.

A longitudinal, prospective community study based in Norway and Denmark, examined the risks of developing dementia in a large community based cohort of non-demented subjects with Pd and in a random sample of elderly controls[3]. Parkinson's disease was diagnosed and characterised by means of interview, clinical examination and laboratory tests. Assessment of motor symptoms was carried out using the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn and Yahr scale. Cognitive assessment included subject and carer interviews, the Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised (DSM-III-R) plus three cognitive rating scales - the Mini Mental State Examination (MMSE), Gottfries Bråne and Steen, and the Mental subscale of the UPDRS. Hallucinations were assessed using the Thought Disorder item of the UPDRS. The presence and severity of depression was also assessed. Some mild cognitive impairment was allowed (i.e. MMSE<29) but not DSM criteria for dementia. The subjects underwent follow up evaluation between 4.2 and 5 years. Individuals with a MMSE <16 underwent a further battery of neuro-psychological testing which included tests of executive and visuo-spatial function. 507 controls and 36 Pd sufferers died during follow up. 1908 (from 3062) controls and 130 (from 171) Pd sufferers completed the follow up. The Pd group was slightly younger than the control group (69.8 years vs 72.4 years) and had undergone more years in formal education (39% Pd sufferers had <8years education vs 64% controls). However there was no significant difference in gender ratio or MMSE score between the two groups at baseline. 33% (43) of the Pd sufferers developed dementia, as compared to 10% of the controls. 2 of the 43 had only possible dementia but even excluding those 2, the incidence of dementia in Pd was 95.3 per 1000 person years (95% CI of 72-129) as compared to 25.5 per 1000 person years in controls (95% CI 22.5-28.5). Death led to 36 of the Pd sufferers being lost to follow up, and this may have caused a relative underestimate of the true incidence of Pd dementia. The study concluded the risk of developing dementia in Pd was nearly six times higher than in the general elderly population (OR 5.9; 95% CI 3.9-9.1) after adjustment for age, sex, and educational background. To adjust for the greater drop out rate due to refusals and deaths in the control group, analysis after two years follow up showed the OR remained just as high (5.6 95% CI 3.6-8.6). This study shows that Pd sufferers are at high risk of developing dementia.

What factors are associated with the development of dementia in PS? Multivariate analysis showed subjects who became demented were older, had longer disease duration, a lower MMSE score at baseline, higher thought disorder score, more severe parkinsonism and needed higher L-dopa doses at baseline than those who remained non demented. The latter seems to reflect more severe motor symptoms. A declining response to L-dopa has been reported as a determinant of dementia in other studies[9,10] though not in this study. The mean daily dose of L-dopa at follow-up in this study did not differ from the non-demented group. A trend toward depression was also found in those who became demented. Only the Hoehn and Yahr stage and UPDRS motor score were highly inter-correlated, and independent risk factors at baseline for dementia development in Pd were: age; MMSE < 29; and Hoehn and Yahr stage > 2. There did not appear to be an association between PS type and dementia in this particular study. Other studies have also found that demented PS sufferers are older, with worse motor function.11,12 Depression, bradykinesia and postural instability - dominant parkinsonism (as opposed to tremor-dominant) - and symmetrical onset of motor symptoms have also been put forward as risk factors for cognitive decline[10,13-15].

Neuropathology in the dementias of parkinsonism

Individuals with PS have abnormally functioning basal ganglia connections and pathways. The cerebral cortex and striatum are connected with a complex series of neuronal circuits. These involve the putamen, caudate nucleus, substantia nigra, thalamus, globus pallidus externa, globus pallidus interna, subthalamic nucleus, reticular formation, cerebral cortex, cerebellum, cranial nerve nuclei and spinal cord. A motor circuit in which the putamen receives connections from the sensorimotor cortex is responsible for initiation, sequencing and modulation of movement and control of voluntary and involuntary movements[16]. A cognitive circuit also exists in which the caudate nucleus receives connections from the prefrontal cortex and visual and auditory areas of the temporal and parietal cortices. This is involved in a number of higher cognitive abilities such as learning, short and long term memory, problem solving and attention[17,18]. The substantia nigra provides dopaminergic innervation to the striatum; thus degeneration of this nucleus, as seen in PS, results in severe dopamine reduction and cell loss in the striatum[17]. This interferes with the normal functioning of these motor and cognitive circuits[16,19,20]. In one small study the number of neurones in the lateral part of the substantia nigra showed a negative correlation with the severity of rigidity and hypokinesia, whilst the degree of dementia was significantly correlated with neuronal loss in the medial part of the substantia nigra[21].

It appears Lewy bodies present as neurones are lost. Lewy bodies (LBs) are spherical, intracytoplasmic, eosinophilic, neuronal inclusions composed of neurofilament proteins. It has been suggested that their function is to eliminate damaged proteins from cells as part of a protective response to stress[6]. Two syndromes related to PS and cognitive impairment - Parkinson's disease (Pd) and Dementia with Lewy bodies (DLB) - merit further consideration. LBs can be found in both and there is a correlation between the density of LBs in the frontal and temporal lobes and hippocampus, and cognitive impairment in both Pd and DLB[22,23]. Although there is overlap between the pathological changes of Pd, DLB and Alzheimers disease (AD), subjects with Pd have LBs predominantly in the brainstem nuclei and the substantia nigra. Patients with DLB have LBs predominantly in the cortices and substantia nigra[6]. The underlying neurobiological basis of Pd and DLB however is essentially similar; one study found that the distribution of LBs did not differentiate between DLB and Pd[24]. The formation of LBs begins with the accumulation of the presynaptic protein alpha-synuclein (thought to be important in the production of synaptic vesicles)[25,26]. Cytoskeletal proteins become trapped within the alpha-synuclein aggregates. Ubiquitin and parkin processing is also abnormal[27]. One aim in the development of preventative treatment will be to modify abnormal processing of alpha-synuclein and related proteins. Depletion of dopamine is the main neurochemical abnormality in PS but other neurotrasmitters are also affected, including noradrenaline, serotonin and acetylcholine. A subset of nicotinic acetylcholinergic receptors has been shown to be reduced in several neurodegenerative disorders including AD (eg in the parahippocampal gyrus), Pd and DLB (eg in the substantia nigra, caudate and putamen)[28]. Reductions in cortical choline acetyltransferase in DLB correlates with the severity of cognitive impairment[6] and visual hallucinations[29] though this is not the sole explanation for the observed neuropsychiatric features. In DLB a selective reduction in presynaptic cholinergic activity in the reticular nucleus of the thalamus has also been shown which relates to disorders of consciousness as well as hallucinations, both prevalent in this disorde[28]. Abnormality in the high affinity nicotinic receptor subtype in the temporal association cortex is also related to disorders of consciousness. In Pd and DLB muscarinic M1 binding is elevated and not uncoupled to the same extent as in AD. This is relevant to the response to cholinergic therapy seen in these conditions. The M1 receptor is more elevated in individuals with delusions than those without them. This may be due to the loss of presynaptic cholinergic activity which leads to over compensation in the M1 receptor function[6,30].

Clinical features

Consensus guidelines describe the classic triad of symptoms of DLB, as distinct from "Pd plus dementia"[31,32]. The three components of this triad are fluctuating consciousness, visual hallucinations and parkinsonism. At least two of these components, plus dementia, need to be present to make the diagnosis of probable DLB; and one of these components and dementia for possible DLB. The fluctuation in consciousness can be particularly difficult to detect and this could well be the reason for the significant inter-observer variability in the diagnosis of this condition. Autopsy[33] and epidemiological studies[34] suggest that DLB may account for up to 20% of all cases of dementia. Features supportive of the diagnosis of DLB include autonomic dysfunction, falls, syncope, neuroleptic sensitivity, systematised delusions, hallucinations in other modalities, depression and rapid-eye-movement (REM) sleep and behavioural disorders[6]. Autonomic dysfunction may be prominent, contributing to postural hypotension and falls, which are very common in DLB. In those with PS dementia, and particularly DLB, there is extreme sensitivity to worsening of their parkinsonism by neuroleptics - even by the so-called "atypical neuroleptics" (AN). The sensitivity manifests itself by a severe and rapid decline in motor symptoms. This can be so severe that patients stop eating and drinking, and it is associated with a 2-3 fold increased risk of death. Only moderate dopamine reduction and neurone loss is seen in the substantia nigra in DLB sufferers when compared to those with Pd. Dopamine D2 receptors are increased, at least initially in Pd, as a result of 'up regulation'. But by contrast, D2 receptors are reduced in DLB[35]. This may be part of the reason why patients with DLB exhibit such bad reactions to neuroleptics. Up to 80% of individuals with DLB suffer neuropsychiatric symptoms, particularly visual and auditory hallucinations. These can be very distressing. Visual hallucinations are often three-dimensional, in colour and involve detailed images of people or animals -frequently dysmorphic or with disconnected body parts[6]. Simple global measures of cognition (eg MMSE) are usually equivalent to those of AD at a comparable stage. But DLB sufferers do particularly badly on visual perceptual, spatial, attention and learning tasks, and the decline in cognition is often faster than that seen in AD and vascular dementia[6]. Despite this, individuals with DLB are often better at motor tasks than Pd sufferers.

DLB and PD plus dementia - 1 disease or 2?

Controversy persists as to whether Pd and DLB represent single entities or two separate diseases. Some would argue that a useful approach is to think of a spectrum of presentation of LB disease with motor Pd at one end and DLB at the other.6 The clinical and pathological features outlined above suggest that Pd and DLB can be regarded as two separate entities within a clinical context. To illustrate this hypothesis, a neuro-pathologist, blinded to clinical diagnoses, was asked to evaluate brains which had been labelled as having pure LB pathology, and to determine LB distribution and frequency[4]. The neuro-pathologist was also asked to evaluate a control group of brains with AD changes. Then clinicians, blinded to LB distribution and frequency, determined consensus clinical diagnoses using published criteria for DLB, AD and Pd. Only 11 of the 66 brains were selected by the neuro-pathologist as having largely pure LB disease: an indication of the degree of overlap between the pathologies of AD and LB disease. But clinical features separated cases into two groups and these groups were distinguishable mainly by the time course of clinical symptoms.

One group presented with parkinsonism and had variable development of dementia. If dementia occurred, it appeared late in the course of the Pd. Delusions occurred in 2 out of 5, onset occurring at 14 and 21 years into the disease course respectively. Depression, falls, visual hallucinations and fluctuation in cognition occurred in some, but again only late in the course of the disease. The second group was more heterogeneous. All presented with dementia or pyschosis, and features of parkinsonism were variably present. These individuals had a shorter disease course, dying within 10 years of disease onset. The clinical features supporting a diagnosis of DLB were present in all cases, and always appeared early in the course of the disease. These individuals were also older at disease onset. Based on the known LB pathology and the presentation with cognitive and behavioural abnormalities, cases in the second group were most compatible with a diagnosis of DLB.

In Aarsland's community based prospective study investigating the risks of developing dementia in Pd it was presumed that AD and vascular dementia would occur with similar frequencies in the Pd and control groups[3]. The association of dementia with motor symptoms in that study suggests that motor and cognitive symptoms share common mechanisms. However this study also found that mild cognitive impairment predicts subsequent dementia. It may be that an increasing density of cortical LBs over time in a subset of patients with PD leads to dementia. Perhaps age at onset influences the clinical course of Pd: in Richard's study the group with clinical features of Pd were generally younger at disease onset[4]. There may be interactions between the pathological disease process and normal ageing, as variations in clinical presentation probably reflect selective involvement of different subsets of neurones in an age-dependent manner[6]. Whether or not Pd and DLB are one disease or two, it does seem certain there are two subsets.

Investigations of DLB

Diagnosis of DLB is based on history and examination according to consensus guidelines. Some investigations may be performed in the course of clinical evaluation or research. No diagnostically useful tests have yet been established although cerebro-spinal fluid levels of Aß42 protein have been reported to be decreased and Tau protein to be normal in DLB as opposed to AD.36 The electroencephalogram is often abnormal with a greater degree of background and temporal slowing than in AD. It usually also exhibits the reduced movement potential seen in Pd[6]. The main characteristic of DLB seen on brain imaging is relative preservation of the hippocampus and medial temporal lobe compared with in AD[37]. Other measures such as degree of ventricular enlargement, rate of volume loss over time using serial MRIs, and white matter lesion count, are similar in DLB and AD[37-39]. Positron emission tomography (PET) and single-photon emission tomography (SPECT) show patterns similar to AD. However pronounced hypoperfusion in the posterior parietal cortex and hypometabolism of the occipital and visual cortex is seen in DLB when compared to AD, whereas again temporal lobe perfusion is relatively well preserved[40,41].

Managing psychotic symptoms in dementia with parkinsonism

With any individual exhibiting psychotic symptoms, exacerbating factors such as infections and drugs must be sought and eliminated. For those treated with anti-parkinson's medication, dose reduction or even withdrawal should be considered, at least on a trial basis. If there is no improvement then a cautious trial of an anti-psychotic agent may be considered. Initial optimism was invested in newer, so called, 'atypical neuroleptics' (AN), especially with regard to DLB where the severe sensitivity reaction associated with 'conventional' neuroleptics might be avoided. Unfortunately an increasing number of case reports indicate that neuroleptic sensitivity does still occur, especially as the dose is increased[42-45]. Another therapeutic option is to employ non-neuroleptic medication. Low dose clonazepam (0.5-1 mg for example) is particularly useful for sleep disturbance[43]. Four atypical neuroleptics have most research behind them - Clozapine, Risperidone, Olanzapine and Quetiapine. Clozapine works rapidly and has been shown by a meta-analysis to have an 85% response rate in terms of reduction in psychotic symptoms. A placebo-controlled trial showed no worsening of motor symptoms and there is some evidence that tremor improves[46,47]. However the risk of agranulocytosis means that patients need very regular monitoring of their blood count: this can be inconvenient. Only very small doses (25 mg/day) are needed. Risperidone, even in doses under 1 mg/day, may be associated with worsening motor symptoms though the reports are fewer and somewhat mixed. The situation is similar with Olanzapine but in addition to being associated with worsening motor function, worsening cognition is also recorded[5]. Although Quetiapine is well tolerated at lower doses (<75 mg) studies do show evidence of some decline in motor function, but usually not enough for the drug to have to be discontinued[5,48-50]. The observed decline in motor function may in part be attributable to disease progression[5]. It is important to remember that all these medications can prolong the QT interval on the electrocardiogram (ECG) and an ECG taken before and regularly during treatment is advisable. Patients with DLB psychosis may need higher doses of ANs. This may precipitate a decline in motor function. Despite this, ANs are still probably safer than 'conventional' neuroleptics.

A rational, effective and possibly safer option for the treatment of psychosis in these patients may be to compensate for the cholinergic deficits, with the addition of acetylcholine esterase inhibitors (AChEI). The activity of the cholinergic system is reduced more in DLB as compared to AD[6]. It therefore makes intuitive sense that AChEI will be more effective in treating the cognitive, functional and other abnormalities in DLB compared with in AD. Both Donepezil[51] and Rivastigmine[52,53] have been shown to improve cognition (and MMSE score) in small open label studies, and other small studies have also shown improvements in delirium-like and psychotic features including attention, orientation and frequency, duration and content of hallucinations with Tacrine, Donepezil and Rivastigmine[51-56]. Improvements are seen in the overall Neuropsychiatric Inventory Score (NPI)[52,53]. AChEIs also improve function[51,53], sleep disturbance[53,55] and carer distress[55]. In one multicentre, randomised placebo-controlled trial, 120 subjects with a clinical diagnosis of probable or possible DLB and with a MMSE >10 were treated with Rivastigmine 12 mg or placebo for 20 weeks. Approximately twice as many subjects on Rivastigmine as on placebo (63% compared with 30%) showed at least a 30% improvement from baseline in neuropsychiatric and cognitive tests[57]. The improvements with AChEI in DLB and Parkinson's plus dementia are generally reported as being greater than those achieved with similar doses of these drugs in AD. Sustained improvement in cognition and neuropsychiatric symptoms has been documented for over 2 years of treatment with AChEIs[52]. These improvements seem to occur without significant deterioration of motor function. Drop-outs (generally 10-25%) were usually due to peripheral cholinergic side effects or non-compliance. Titration protocols and dosing were generally the same as for AD[6]. AChEI should probably be the first choice for symptomatic treatment of the core symptoms of DLB, particularly because they do not carry the adverse risks associated with all types of neuroleptics.

Discussion

A combination of clinical discrimination and investigation will usually enable definition of the character of the disease in a PS sufferer with declining cognition. Depression and delirium commonly mimic dementia and are important causes of cognitive impairment. These need to be excluded by a thorough history, examination and appropriate investigation (such as laboratory tests for infection or metabolic disorders). Corroborative history from carers and relatives is essential. Enquiries should focus on other cognitive and behavioural symptoms apart from memory problems, and also risk factors for other dementias such as family history of AD, and vascular risk factors for vascular dementia. DSM criteria for dementia are memory deficit plus: an associated symptom (such as dysphasia or other cognitive dysfunction); an indication that the illness has adversely affected the patient's social or functional capacity; and confirmation the illness is not due to any other psychiatric condition[58]. An accurate determination of timescale of the cognitive decline is also essential. DLB runs a much shorter course than PS plus dementia. DLB sufferers are generally older at the onset of cognitive decline than those with Pd and cognitive impairment, and psychoses are much more prominent early on in DLB when compared to PS[4]. Standard global tests of cognition such as the MMSE, whilst important, may not detect impairments of some cognitive functions and more detailed neuropsychological testing may be required. The psychiatric disturbances are generally more severe in DLB when compared to PS, and those with DLB do particularly badly on tests of visuospatial function when compared to those with AD, vascular dementia or PS[6]. Clinical examination may also lead to the consideration of other causes of dementia such as hypertension and gait abnormality in vascular dementia; or the rarer progressive supranuclear palsy (the patient is unable to move eyes upward). Parkinsonism may be variably present in DLB sufferers and show greater symmetry and reduced resting tremor than seen in Pd although some studies have not confirmed these findings[59-60]. DLB sufferers may also have more severe action tremor, bradykinesia and gait disturbances as compared to those with Pd[61]. Brain imaging is indicated in the investigation of dementia. The rare, but potentially reversible normal pressure hydrocephalus is an important exclusion. Structural and functional imaging shows promise in distinguishing between DLB and AD but are not readily available outside a research setting. DLB should be diagnosed according to consensus guidelines: at least 2 of the triad of fluctuating consciousness, visual hallucinations and parkinsonism, plus dementia need to be present to make the diagnosis of probable DLB and one of this triad plus dementia for possible DLB. Supporting features for a diagnosis of DLB should be sought.

Treatment of this group includes treatment of their parkinsonism, though those with DLB may not respond so well to L-dopa as those with Pd[10,62]. Treatment of any patient with dementia is largely supportive. This must be multidisciplinary and extend to carers. Use of respite care, day centres and local support groups can be invaluable in easing the burden that the illness places on both patient and carers. Treatment plans and advice regarding policies for the future should be sensitively discussed. For example it may be appropriate to discuss the use of advance directives to help maintain a patients autonomy should they later become incapable of expressing their wishes at a particular decision point. Power of attorney may also be awarded to a trusted relative or carer who may then manage the patients affairs once they are incapable of doing do themselves. Drug treatment for the dementia can now be aimed at treating behavioural and psychiatric symptoms, or slowing the progression of cognitive decline. Neuroleptics should be avoided in any patient with possible DLB. For those treated with anti-parkinson's medication, dose reduction or withdrawal should be considered, moving on to a cautious trial of an AN if there is no improvement. Of these Clozapine and Quetiapine are probably the least likely to cause worsening of the parkinsonism, though careful monitoring of the ECG and for Clozapine, the FBC, are necessary[5,10]. Other non-neuroleptic medications may have a role, such as low dose clonazepam for sleep disturbance[10,43]. However, AChEIs do not carry the adverse risks associated with the neuroleptics and should probably be the first choice for symptomatic treatment of DLB both for cognitive and non-cognitive symptoms[6,10]. Treatment of concomitant depression can result in significant functional improvements. Serotonin reuptake inhibitors are favoured over anticholinergic antidepressants such as tricyclics because of their preferable side effect profile.

Conclusion

Dementia is an important cause of morbidity and mortality in PS. Those developing PS are approximately six times more likely to develop dementia than in the general elderly population. Risk factors for develop-

Table 1. Clinical and pathological differences between Dementia with Lewy bodies (DLB) and Parkinson's disease (Pd).

	DLB	Pd plus dementia
Presentation	Usually present with dementia or psychosis.	Parkinsonism
Psychiatric and cognitive features	Appear early. More severe visuospatial dysfunction.	Appear late in the disease course.
Parkinsonism	Often greater symmetry and reduced rest tremor than Pd. Greater degree of bradykinesia and action tremor andfalls tend to be more prominent.	Less symmetrical compared with DLB.
Motor response to L-dopa	Tends to respond less well than Pd.	Good response
Disease duration	Short(,10 years)	Long
Age of onset	Older	Younger
LB distribution	Predominantly in cortices and substantia nigra.	Predominantly in brain stem nuclei and substantia nigra.

ing dementia in PS includes older age, longer disease duration, lower MMSE and more severe parkinsonism. Some of these individuals can be reclassified as having DLB. Whether DLB is really a separate disease or represents one end of a spectrum of LB disease is still debated, although useful discriminators can be deduced with detailed history and clinical examination. Table 1 summarises the salient differences between DLB and Pd plus dementia. Some of the atypical neuroleptics, notably Clozapine and Quetiapine, can be useful in treating psychotic symptoms in these patients, although there is now promising evidence for the efficacy and safety of AChEIs, both in treating the cognitive and non-cognitive symptoms afflicting these individuals. Further large randomised controlled trials are necessary.

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Increased 4-Hydroxy-2-Nonenal, a Major End Product of Lipid Peroxidation, in the Brains of two Senescence Accelerated Mouse Strains, SAMP8 and SAMP10

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Abstract

Senescence accelerated mouse strains (SAMP8 and SAMP10) have been established as animal models of aging. In contrast, SAMR1 is a closely related strain with a normal aging process. 4-hydroxy-2-nonenal (HNE), a major end product of lipid peroxidation, plays an important role in the cytopathological effects during oxidative stress in vivo. Using an immunocytochemical method, we observed that in the brains of SAMP8 and SAMP10 mice, increased levels of HNE-modified proteins were detected relative to SAMR1 brains. Intense HNE-modified proteins were detected in the cytoplasm of neurons, neuropil, blood vessels in olfactory lobe, cortex, hippocampus, septum, paraventricle areas, thalamus, hypothalamus, brain stem and in the Purkinje cells of cerebellum in SAMP8 and SAMP10 mice. Our studies suggest that (1) oxidative stress is present in SAMP8 and SAMP10 mice, and might play a role in the aging process; (2) HNE might contribute to the cytopathological changes in the brains of these mice.

Keywords: *SAMP8, SAMP10, SAMR1, HNE, Oxidative Stress, Lipid Peroxidation.*

Introduction

Animal models of accelerated senescence were developed in inbred mouse strains derived from an inadvertent cross between AKR mice and an unknown strain or strains. The strains prone to signs of accelerated senescence are referred to as senescence-accelerated mouse prone (SAMP)[1,2]. Some inbred strains derived from the same cross are resistant to early senescence, and these are termed SAMR. There are several senescence accelerated prone strains and four strains resistant to accelerated senescence. SAMP strains share a number of generalized signs of early senescence, which include ruffled coat, lordokyphosis, perioptic skin lesions, reduced activity and shortened life span[1,3]. In addition, each SAMP strain has a strain-specific clinical and/or pathological phenotype. These clinical findings include early deficits in learning and memory, cataract, brain atrophy, senile osteoporosis and degeneration of the temporomandibular joint. In the SAMP series, SAMP8 and SAMP10 mice show early onset and rapid advancement of senescence, which is revealed by analysis of aging dynamics, such as survivorship curves, and a grading system for specific age related changes[3]. They also exhibit a significant age-related deterioration of memory and learning abilities for passive and active avoidance tasks compared to SAMR1 mice[4]. Compared to the average life span for the four SAMR strains, the life spans of SAMP strains were 43-85% as long, with seven of the nine strains having life spans less than 61% of the average SAM R value[1,2].

The mechanism(s) of induction of accelerated aging and age-associated deficits in learning and memory in SAMP8 and SAMP10 mice is still unclear. Analysis of the expression of endogenous murine Leukemia viruses (MuLV) in the brains of senescence-accelerated mice (SAMP8) and the relationship between expression and brain histopathology, Jeong et al. (2002)[5] suggested that MuLV could play an

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important role in the brain aging processes in SAMP8 mouse. Several lines of evidence also indicate that oxidative stress may play an important role in various pathological states including cancer, neurodegeneration, atherosclerosis, diabetes, cancer, and rheumatoid arthritis, as well as in drug-associated toxicity, post-ischemic reoxygenation injury, and aging[6]. It has been reported that SAMP8 mice exhibit oxidative stress in the brain including a decrease in the relevant electron paramagnetic resonance (EPR) parameter, a decreased glutamine synthetase activity and an increased protein carbonyl content compared with the values in SAMR1 mice[7]. It has been found that 4-hydroxy-2-nonenal (HNE), a major end product of lipid peroxidation, plays an important role in the cytopathological effects observed during oxidative stress *in vivo*[8]. In the current study, using an immunocytochemical method, we investigated the presence and/or the localization of HNE-modified proteins in the brains of SAMP8, SAMP10 and SAMR1 mice. We hypothesize that HNE might exist in the brains of SAMP8 and SAMP10 mice and play an important role in the aging process and that HNE might contribute to the oxidative stress in the brains of senescence-accelerated animals.

Materials and Methods

Animals

The SAMP8/Ta, SAMP10/Ta and SAMR1/Ta strains were kindly provided by Toshio Takeda (Kyoto University, Kyoto, Japan) and have been maintained in our animal colony for the past 10 years as inbred strains. AKR/J and C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbour, ME, USA). Pathogen-free SAMR1 and SAMP8 animals were obtained subsequently from Drs. J.F. Flood and J.E. Morley (1994)[9] and have been housed in Thoren cages in a clean facility separate from the main animal colony. The pathogen-free mice have been bled and checked serologically for common murine pathogens by the serological testing service of Charles River Laboratories. These mice have remained pathogen free. All mice are on a 12-hr light, dark cycle. Animals are fed and watered *ad libitum*. There were at least seven mice in each group. The experiments were approved by the Institutional Animal Care and Use Committee in our Institute.

Tissue Preparation

All mice (about 15 months old) were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal) and were perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The brains were dissected. Brain specimens were immersion fixed in the same fixative overnight and sectioned into seven tissue sections corresponding to levels 180, 250, 340, and 500 of Sidman's Mouse Brain Atlas[10]. Tissue specimens were then processed into paraffin blocks and sectioned into 7-μm-thick histological sections. The sections were mounted on poly-L-lysine coated slides and allowed to air-dry overnight at 37°C.

Immunocytochemistry

The sections were dewaxed in xylene, placed in 100% ethanol, and then were rehydrated through graded ethanol solutions prior to being rinsed in PBS. Immunostaining was performed by an immunodetection kit for detecting mouse primary antibodies on mouse tissue (M.O.M. kit and/or Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA). Endogenous peroxidases were quenched with 3% H_2O_2 in 100% methanol for 15 min. Nonspecific sites were blocked by exposure to mouse Ig blocking solution in PBS; incubation was for 30 min at room temperature. The sections were then incubated overnight at 4°C with mouse monoclonal anti-HNE-protein (1:200) (a gift from Koji Uchida, Nagoya University, Nagoya, Japan). As negative controls, the primary antibody was replaced by non-immune rabbit serum or mouse serum. For the supersensitive alkaline phosphatase test, after reaction with the primary antibody and rinses, the sections were incubated sequentially at room temperature with biotinylated secondary antibody (1:100), alkaline phosphatase-conjugated streptavidin (1:100) and chromogen substrate (BioGenex Laboratories, San Ramon, CA)[11]. Some of the slides were counterstained with cresyl violet.

Results

Table 1 shows the comparison of HNE-immunostaining intensity in brain regions among SAMR1, SAMP8 and SAMP10 mice. In the brains of SAMP8 and SAMP10 mice, increased levels of HNE-modified

Table 1. Comparison of HNE-immunostaining intensity in brain regions in SAMR1, SAMP8 and SAMP10 mice.

Brain regions	SAMR1	SAMP8	SAMP10
Cortex	+	+++	+++
Septum	+	++	++
Hippocampus	+	+++	+++
Thalamus	+	+++	++
Hypothalamus	+	++	++
Brain Stem	+	+++	++
Cerebella	+	+++	+++

Note: +: few stain, ++: moderate stain, +++: strong stain.

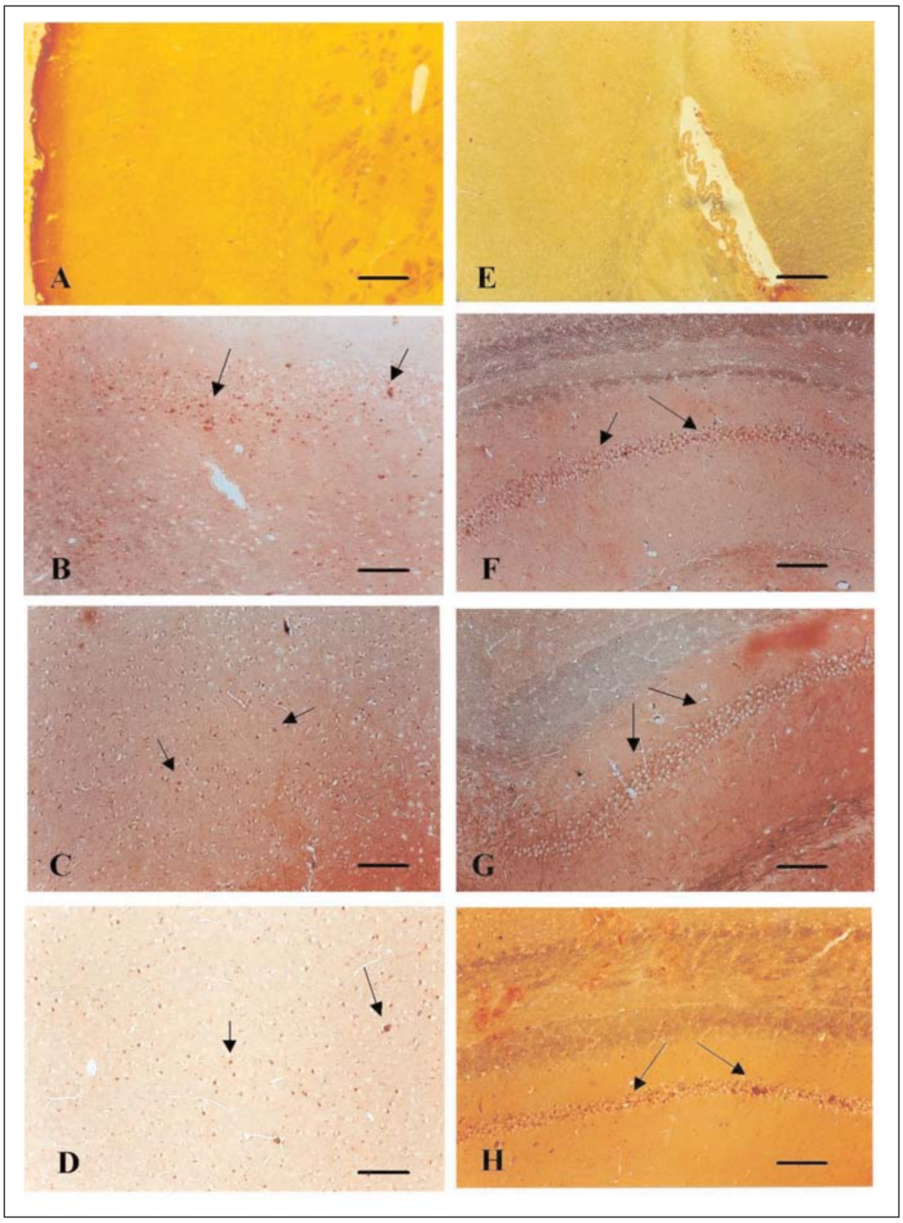


Figure 1.
A. Very few and weak HNE-immunostaining neurons were found in the cortex (layer I to layer VI) of SAMR1 mice. Bar: 200µm.
B. Increased number and intensity of HNE-immunostaining neurons (arrows) in the cortex (frontoparietal cortex, layer II, motor area) of SAMP8 mice. Bar: 100µm.
C. Increased number and intensity of HNE-immunostaining neurons (arrows) and blood vessel cells in the cortex (layer III-VI) of SAMP8 mice. Bar: 100µm.
D. Increased number and intensity of HNE-immunostaining neurons (arrows) in the cortex (layer III-VI) of SAMP10 mice. Bar: 100µm.
E. Very few and weak HNE-immunostaining neurons were found in the cortex, paraventriclar area and hippocampus of SAMR1 mice. Bar: 200µm.
F. Increased number and intensity of HNE-immunostaining neurons (arrows) and blood vessel cells in the hippocampus (CA2) of SAMP8 mice. Bar: 100µm.
G. Increased HNE-immunostaining neurons (arrows) and blood vessel cells in the hippocampus (CA1) of SAMP8 mice. Bar: 100µm.
H. Increased number and intensity of HNE-immunostaining neurons (arrows) in the hippocampus (CA2) of SAMP10 mice. Bar: 100µm.

proteins were detected relative to SAMR1 control brains. Intense staining of HNE-modified proteins was detected in the cytoplasm of neurons, neuropil, and blood vessels in olfactory lobe, cortex, hippocampus, septum, paraventricle areas, thalamus, hypothalamus and brain stem; staining was also seen in the Purkinje cells of cerebella in SAMP8 and SAMP10 mice.

Very weak HNE-immunostaining of a few neurons was found in the cortex of SAMR1 mice (Fig. 1A). In contrast, there were many more HNE-immunostaining cells in the cortex of SAMP8 and SAMP10 mice than in SAMR1 mice (Fig. 1B-1D). Intense HNE-immunostaining neurons were found in all layers of cortex, particularly layers 1 and 2 (Fig. 1B). HNE-immunoreactivity was found in cytoplasm and close to the nucleus of the neurons (Figs. 1B-1D), as well as in blood vessel cells in the cortex of SAMP8 and SAMP10 mice (Figs. 1B-1D).

In hippocampus, only weak HNE-immunostaining was found in neurons of the paraventricular area and hippocampus of SAMR1 mice (Fig. 1E). Increased HNE-immunostaining of both neurons (arrows) and blood vessel cells was found in the hippocampus of SAMP8 (Fig. 1F and 1G) and SAMP10 mice (Fig. 1H). Most of the HNE-immunostaining was found in the granular layer and pyramidal cell layer of hippocampus and in the dentate gyrus. There were more HNE-immunostaining blood vessel cells in the hippocampus complex of SAMP8 and SAMP10 mice than in SAMR1 mice. More intensive HNE-immunostaining neurons were found in the fimbria hippocampus, stria terminalis and stratum subependymale ventriculi lateralis of SAMP8 mice than SAMR1 mice (Fig. 2A and 2B). Increased HNE-immunostaining of neurons and blood vessel cells was found in the thalamus (Fig. 2D), the hypothalamus (Fig. 2E), putamen

(Fig. 2F), brain stem (Fig. 2G), and parocellular reticular nuclei (Fig. 2H) of SAMP8 mice compared to SAMR1 mice. Within the thalamus, HNE immunoreactivity was detected in neurons in several regions including the lateral habenular nucleus and the paraventricular thalamic nucleus. SAMP8 and SAMP10 mice have increased HNE immunoreactivity in the hypothalamus, especially in the paraventricular hypothalamus and preoptic region.

Very few and weakly stained HNE-neurons were found in the brain stem areas of SAMR1 mice (Fig. 3A). In contrast, the number and intensity of HNE-immunostaining neurons (arrows) were increased in the areas of the interpositus cerebellar nuclei, the gigantocellularis nuclei and the paragigantocellularis reticular lateralis nuclei of SAMP8 mice (Figs. 3B-3D). There were more intensive HNE-immunostaining neurons in the cerebella and paraventricular areas and in Purkinje cells (arrows) in SAMP8 and SAMP10 mice (Figs. 3F and 3G) compared to SAMR1 mice (Fig. 3E). Within the olfactory tubercle, the most striking feature was the intense staining of the neurons in the internal plexiform layer of olfactory bulb in SAMP8 and SAMP10 mice. The HNE-immunostaining was also found in blood vessel cells (Fig. 3H).

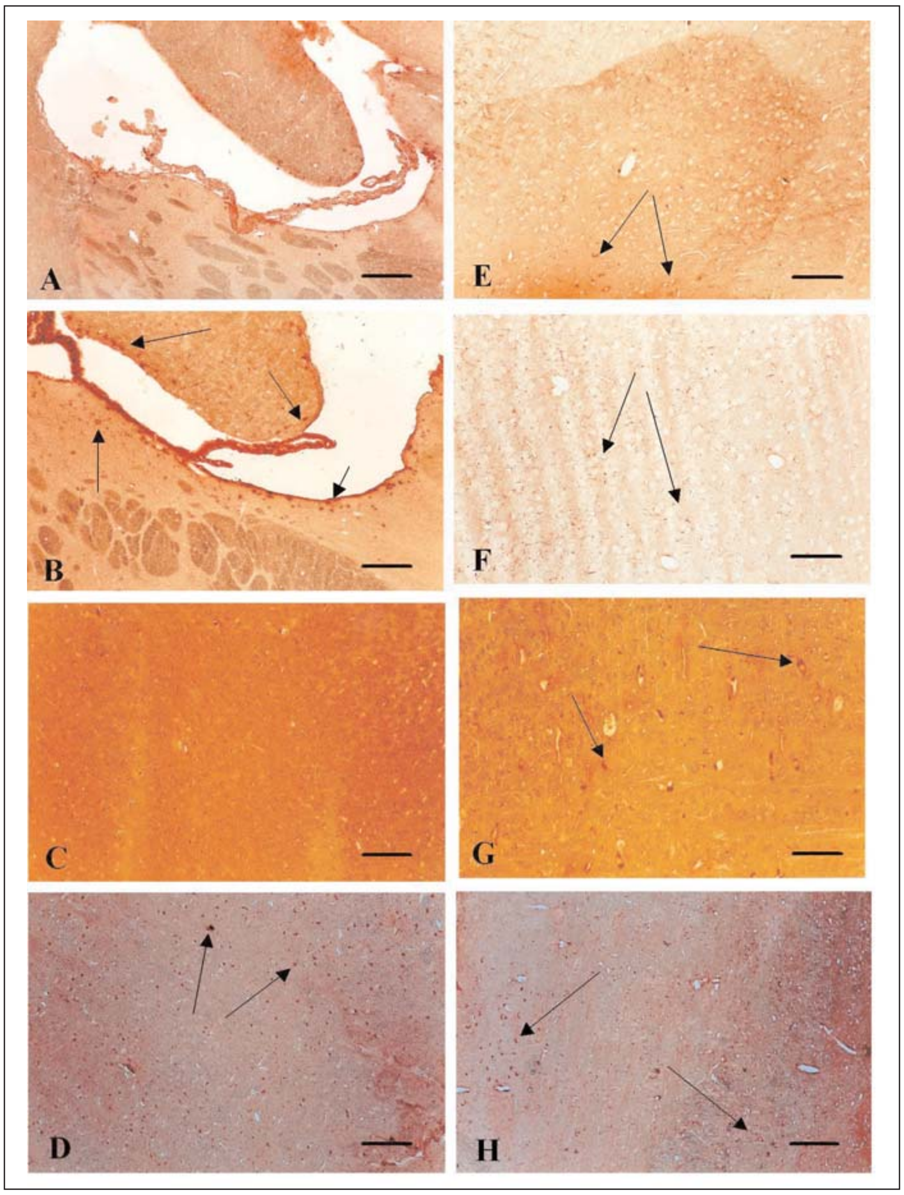


Figure 2.

A. Very few and weak HNE-immunostaining neurons were found in the fimbria hippocampus, stria terminalis and stratum subependymale ventriculi lateralis of SAMR1 mice. Bar: 200µm.

B. Increased number and intensity of HNE-immunostaining neurons (arrows) were found in the fimbria hippocampus, stria terminalis and stratum subependymale ventriculi lateralis of SAMP8 mice. Bar: 200µm.

C. Very few and weak HNE-immunostaining neurons were found in the thalamus of SAMR1 mice. Bar: 100µm.

D. Increased number and intensity of HNE-immunostaining neurons (arrows) in the thalamus of SAMP8 mice. Bar: 100µm.

E - H. Increased number and intensity of HNE-immunostaining neurons (arrows) and blood vessel cells were found in the hypothalamus (E), putamen (F), brain stem (G), and parocellular reticular nuclei (H) of SAMP8 mice. Bar: 100µm.

Discussion

The mechanism(s) of induction of accelerated aging and age-related deficits in learning and memory in SAMP8 and SAMP10 mice is still unknown. Our results indicate very low levels of HNE in brains of SAMR1 control mice and significantly increased levels in brains of SAMP8 and SAMP10 mice. The results suggested that increased HNE might be related to the loss of memory and accelerated aging processes seen in SAMP8 and SAMP10 mice. Takemura et al., (ref. 12) reported a faster age-related increase in the central nervous system of amyloid beta protein (AP)-like immunostaining with polyclonal antibody to AP in SAMP8 mice compared to age-matched SAMR1 control mice. The areas in the brains of SAMP8 mice showing the greatest amount of AP labeling were the cingulate cortex, hippocampus, septum, and brain stem. The distribution of amyloid beta protein in SAMP8 mice is very similar to the distribution of HNE-modified proteins in SAMP8 and SAMP10 mice

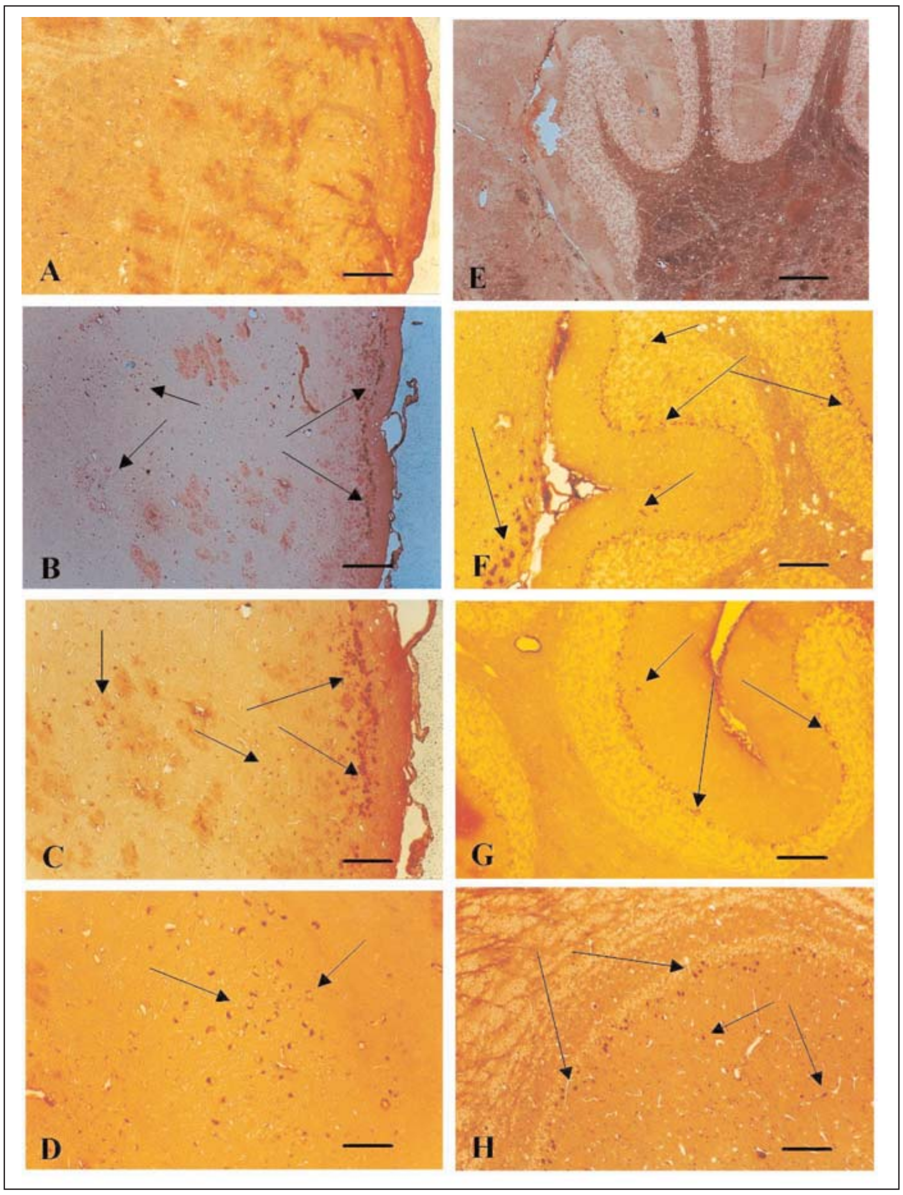


Figure 3.
A. Very few and weak HNE-immunostaining neurons were found in the brain stem areas of SAMR1 mice. Bar: 100µm.
B - C. Increased number and intensity of HNE-immunostaining neurons (arrows) were found in the areas of the gigantocellularis nuclei and the paragigantocellularis reticular lateralis nuclei of SAMP8 mice. B. Bar: 200µm. C. Bar: 100µm.
D. Increased number and intensity of HNE-immunostaining neurons (arrows) were found in the areas of the interpositus cerebellar nuclei of SAMP8 mice. Bar: 50µm.
E. Very few and weak HNE-immunostaining neurons were found in the cerebellar and paraventricular areas of SAMR1 mice. Bar: 200µm.
F. Increased number and intensity of HNE-immunostaining neurons and Purkinje cells (arrows) were found in the areas of paraventricule and cerebella of SAMP8 mice. Bar: 100µm.
G. Increased number and intensity of HNE-immunostaining neurons and Purkinje cells (arrows) were found in the cerebella of SAMP10 mice. Bar: 100µm.
H. Increased number and intensity of HNE-immunostaining neurons (arrows) and blood vessel cells were found in the olfactory bulb of SAMP10 mice. Bar: 100µm.

reported in this study. Our results show that intense HNE-modified proteins were detected in the cytoplasm of neurons, neuropil, and blood vessels in olfactory lobe, cortex, hippocampus, septum, paraventricle areas, thalamus, hypothalamus, brain stem and in the Purkinje cells of cerebellum in SAMP8 and SAMP10 mice. In fact, it has been suggested that lipid peroxidation via free radical injury may be involved in amyloid deposits seen in Alzheimer amyloidosis[13]. Our results supported the hypothesis that lipid peroxidation may occur in every type of amyloid deposit of localized and systemic amyloidosis as suggested by Ando et al. (1998)[13]. It has also been reported that several perivascular cells in the brains of Alzheimer's disease patients reacted with antibody against HNE[13]. Increased HNE-modified proteins in perivascular cells in brains of SAMP8 and SAMP10 mice suggested that these cells may undergo oxidative stress and may contribute to amyloid formation, as suggested by Ando et al. (1998)[13].

Considerable experimental evidence supports the idea that aging in general, and aging of the central nervous system in particular, may be in part related to damage inflicted by oxygen free radicals and their intermediates[11,14-16]. It has been found that there is an age-related increase in the content of thiobarbituric acid-reactive substances (TBARS) as well as low glutathione content in SAMP8 mice[17]. It was also reported that oxidized proteins accumulate in the brains of SAMP8 mice more rapidly than in SAMR1 mice[7]. The brain is highly vulnerable to oxidative damage because of its high oxygen utilization, high concentrations of polyunsaturated fatty acids and of transition metals such as iron, combined with low concentrations of cytosolic antioxidants[18]. Lipid peroxidation of cell membranes, as well as oxidative damage to proteins and DNA, have been proposed as explanations for age-associated functional deficits in many organs including the brain[19].

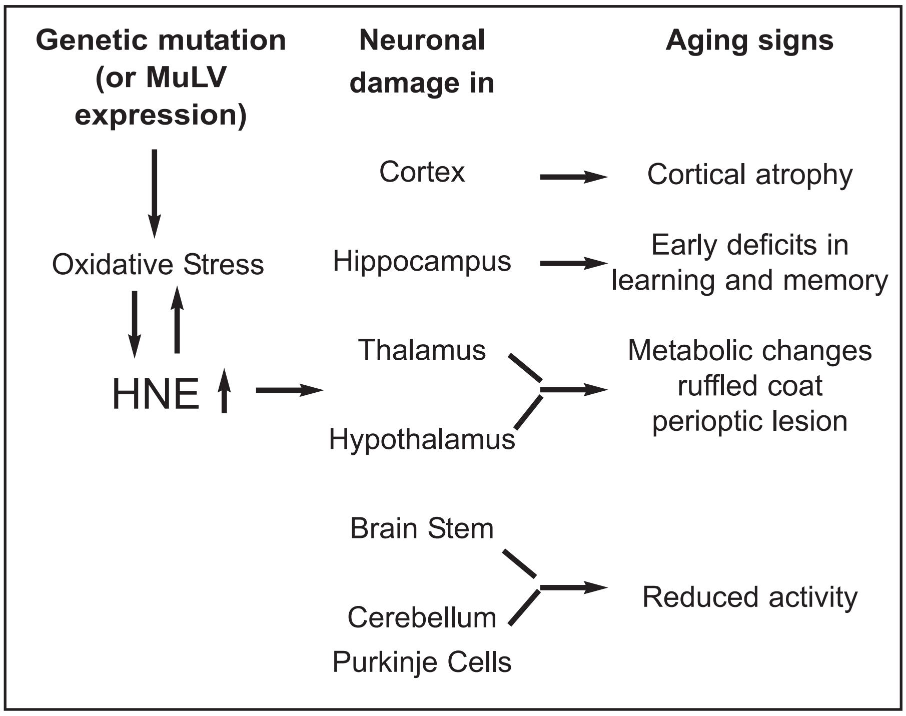
Lipid peroxidation is the consequence of the production and the propagation of free radical reactions primarily involving membrane polyunsaturated fatty acids and has been implicated in the pathogenesis of numerous disease processes[6]. The peroxidative breakdown of polyunsaturated fatty acids has also been implicated in the pathogenesis of many types of cell injury and especially in the cell damage induced by several toxic substances[20]. Among these are the haloalkanes, carbon tetrachloride, trichlorobromomethane, chloroform, dibromoethane, and halothane; in addition, paracetamol, bromobenzene, iron, bipyridyl compounds, allyl alcohol, and in some instances, ethanol have been shown to stimulate lipid peroxidation[21]. It has been reported that aldehydes generated endogenously during the process of lipid peroxidation are causally involved in most of the pathophysiological effects associated with oxidative stress in cells and tissues[22]. Among the lipid peroxidation-derived aldehydes, HNE is believed to be largely responsible for the cytopathological effects observed during oxidative stress in vivo[22]. HNE plays a role in inhibition of protein and DNA synthesis, inactivation of enzymes, stimulation of phospholipase C, and reduction of gap-junction communication. HNE modulates the expression of various genes, including c-myc and globin genes[23], procollagen type I[24], aldolase reductase genes[25], c-myb[26] and transforming growth factor 1 gene[27]. HNE treatment of rat liver epithelial cells (RL34) resulted in depletion of intracellular glutathione (GSH) and in the formation of protein-bound HNE in plasma membranes. In addition, HNE strongly induced intracellular peroxide production and activated stress signal pathways such as c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) in these cells[8]. HNE triggers cellular signaling pathways, leading to activation of activator protein-1 (AP-1)[8,28]. It has also been found that HNE induces the expression of glutathione S-transferase (GST-P), the enzyme that catalyzes the conjugation of reactive chemicals with glutathione and plays an important role in protecting cells against oxidative injury[29].

HNE is highly neurotoxic and increased levels are found in the brains of patients with Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS)[13,30-32]. HNE is also increased in the brain after ischemia injury[33]. HNE is highly reactive and can combine with multiple cellular targets[22], and has an ability to affect the proteasome[34]. The effect of HNE on the proteasome could lead to increased levels of unprocessed but ubiquitinated proteins[35,36]. Growing evidence suggests that increased levels of ubiquitinated or unprocessed proteins render cells sensitive to oxidative stress and apoptotic death[37,38]. Treatment of both NT-2 and SK-N-MC cell lines with HNE led to apoptosis. HNE association with the proteasome leads to increased levels of protein carbonyls and ubiquitinated proteins, and to decreased proteasomal function. There was also decreased metabolic activity, cytochrome c release and activation of caspase-3, followed by apoptotic changes including chromatin condensation, cell shrinkage and DNA fragmentation and cell death[39].

It has been reported that the expression of endogenous MuLV is increased in the brains of SAMP8 mice compared to those in SAMR1 mice[5,40,41]. Immunocytochemistry and electron microscopy showed the colocalization of MuLV with astrocytosis and vacuolation. It has been suggested that MuLV could play an important role in the brain aging processes in SAMP8 mice[41,42]. Further research needs to be done on the relation between increased MuLV expression and increased oxidative stress in SAMP8 mice. It is possible that over expression of MuLV can cause oxidative stress in SAMP8 mice.

Figure 4 shows the possible results of oxidative stress in the brains of SAMP8 and SAMP10 mice. Our results suggest that there was a general increase in HNE-modified protein in the neurons in the brains of SAMP8 and SAMP10 mice compared to SAMR1 mice, particularly in layers 1 and 2 of cortex and in the

Figure 4. Possible Results of Oxidative Stress and Brain Damage in SAMP8 and SAMP10 Mice.



granular layer and pyramidal cell layer of the hippocampus. This may be due to unknown genetic mutation(s) in SAMP8 and SAMP10 mice or over expression of MuLV that causes overall oxidative stress in the brains. The increase in oxidative stress can lead to HNE-modified protein production, which in turn results in higher oxidative stress. The increased HNE-modified proteins in these neurons could alter their normal functions and cause the cortical atrophy found in these animals[43-46]. Because hippocampal neurons are important to memory and learning abilities, the increased HNE-modified proteins in these neurons may play an important role in the early deficits in learning and memory in SAMP8 and SAMP10 mice. The neuronal degeneration in thalamus and hypothalamus may cause neuroendocrine system dysfunction and metabolic changes that lead to general aging signs including ruffled coat, and perioptic skin lesions. We also observed increased HNE-modified proteins in the neurons in brain stem and the Purkinje cells in cerebella of SAMP8 and SAMP10 mice compared to SAMR1 mice. Since Purkinje cells play an important role in motor activity; it is possible that damage to Purkinje neurons by HNE oxidative stress may account for the reduced activity found in SAMP8 and SAMP10 mice. Finally, the increased levels of HNE-modified proteins in neurons may cause neuronal apoptosis, neurodegeneration and brain atrophy in the brains of SAMP8 and SAMP10 mice. Our studies suggest that: (1) oxidative stress occurs in SAMP8 and SAMP10 mice, and that it might play a role in the aging process; (2) HNE contributes to the oxidative damage in the brains of senescence-accelerated animals.

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Mild Cognitive Impairment: Functional Predictors of Progression to Alzheimer Disease

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Abstract

The purpose of this retrospective analysis was to examine whether measures of daily function could predict who would progress to Alzheimer disease (AD) following a diagnosis of Mild Cognitive Impairment (MCI).
Method: ***Analysis of longitudinal data from patients with the diagnosis of MCI was examined. Mini-Mental Status Examination (MMSE) total score and Lawton-Brody Activities of Daily Living (ADL) scales were analyzed to determine whether they could predict progression from MCI to AD within 24 months.***
Results: ***There were no significant differences in age (p>0.05), gender (p>0.05), or baseline MMSE scores (Progressed = 27.0 vs Stable = 27.4, p>0.05). Total ADL scores did not predict conversion. Subscale analysis revealed a significant difference between groups with respect to their ability to plan and prepare meals (p<0.05). This difference was independent of gender.***
Conclusions: ***Subscale IADL items, such as meal preparation, may be predictive of clinical change in people with MCI.***
Keywords: *Mild Cognitive Impairment, Alzheimer disease, Activities of Daily Living, Function, Prognosis, Screening tools.*

Introduction

Mild cognitive impairment (MCI) is an evolving concept, with evolving terminology, in the field of dementia research[1]. Conceptually, it is a transitional phase between normal aging and dementia. It is uncertain whether MCI is necessarily a prodromal dementia state or an independent entity that increases the likelihood of developing dementia. The definition and prognosis of MCI remain contentious. People with objective memory deficits, that are not attributable to reversible causes, are approximately 10 times more likely to develop dementia, particularly Alzheimer disease (AD)[2], than those without MCI. The reported annual progression rate from MCI to AD ranges from 6-25%, whereas it is 1-2% for the normal elderly[3]. A detailed examination of the nosology of MCI has been previously provided by Ritchie[1] and Chertkow[4].

Although MCI has primarily been considered a disorder of memory[5], there are often other cognitive and functional symptoms associated with this disorder[6]. While a memory deficit may be the only aspect of the impairment that is clinically apparent, this may reflect a testing bias, as tests for MCI, based on approaches for early AD, are heavily weighted for short-term memory assessment. A patient-centered approach to MCI recognizes that these deficits often manifest themselves in other ways when the patient is in their home environment.

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As noted by Chertkow[4] a clinical deficit of working memory may mean that the person needs to use a grocery list when one was not needed earlier. This is a functional change that can be attributed to the underlying disorder, and it is often this functional change that prompts the patient and / or their family to seek help.

In order to meet the criteria for MCI, a person cannot be sufficiently impaired in their activities of daily living to meet the criteria of dementia. This definition leaves a fairly wide spectrum of daily function that can be categorized as MCI, ranging from completely normal to relatively impaired. Morris[7] categorized his MCI population into 3 subgroups based on their performance on the six domains (Memory, Orientation, Judgment and Problem Solving, Community Affairs, Home and Hobbies, and Personal Care) of the Clinical Dementia Rating (CDR) scale. All outpatients with a total CDR score of 0.5 were classified as having MCI. Those participants with an isolated memory impairment (equivalent to Petersen's 'amnestic MCI'[3]) were categorized 'MCI – uncertain'. People with impairment of memory as well as impairment in one or two other CDR domains were categorized as 'MCI – incipient', and those with impairment of memory and 3 or more CDR domains were categorized as 'MCI – DAT' (Dementia of the Alzheimer Type). The authors found that those people with more areas of cognitive impairment had a greater rate of progression to Alzheimer disease. That is, patients with deficits in memory plus other domains progressed more quickly to dementia than patients with memory deficits alone. When properly used, functional changes are valuable clinical markers because they are based on a known premorbid level of function, whereas with cognitive assessment, the premorbid level of performance is implied by other variables (i.e. age and education).

Functional impairment in MCI is commonly overlooked, as it is a broadly defined criterion without any accepted objective method for assessment or a clinically relevant cut-off level. It is likely that the confusion about function in MCI stems from its ambigious definition. For the sake of this discussion, it is important to distinguish between Instrumental Activities of Daily Living (IADL's) and Self-Care Activities of Daily Living (SCADL's).

SCADL's are basic, learned daily activities that include Toileting, Feeding, Dressing, Grooming, Physical ambulation, and Bathing[8]. SCADL's are likely to remain unaffected in MCI.

Instrumental ADL's are activities that require higher levels of cognitive function and include Using the Telephone, Shopping, Preparing Meals, Housekeeping, Laundry, Mode of Transportation used, Responsibility for Taking Medication, and Handling Finances. Instrumental ADL's may well be affected to a certain degree in MCI, as in Chertkow's example of beginning to use a shopping list.

Due to the potentially limited degree of functional impairment in MCI, total scale scores tend to dilute any significant effect of the disorder on function. Functional changes in MCI are likely to manifest in a few more complex activities that are different in each individual. They are all learned skills and an individual's capacity for a particular skill will depend on their vocation, role, need, aptitude, or the extent that someone else can provide the skill. For instance, handling complicated finances may be an ideal measure for someone who developed this expertise, however, if this skill was always done by someone else, it would not be an appropriate early indicator of impairment.

Previous papers have demonstrated that function in people with MCI is subtly compromised[9,10,11,12]. Taber[9] demonstrated that self-reported deficits and a discrepancy between self and informant-rated functional deficits were predictive of future diagnosis of AD in the subsequent 2 years. It has also been demonstrated[10] that a careful examination of financial capacity, including objective testing, provided statistically significant difference in overall performance between people with MCI and normal controls.

Clearly, functional capacity in MCI has clinical value, both for initiating appropriate education and supports, as well as potentially having prognostic value. We hypothesized that the manifestation of functional deficits in MCI would affect a higher-order skill. The purpose of this research project was to determine whether careful examination of the level of function of people with MCI could be predictive of progression to AD.

Methods

Participants were recruited from the outpatient population of our Aging Brain Clinic. The Aging Brain Clinic is a memory disorder outpatient clinic in a teaching hospital located in an academic centre of 350,000 people, serving a region of 1.5 million people (200,000 over 65 years of age) in Ontario Canada. The clinic has 2 geriatricians, 2 nurse practitioners, and a team of research staff. Assessments conducted at baseline and follow-up visits include a corroborated medical history, physical exam, assessment of function, affect, cognition, and behaviour. Patients are seen in annual follow-up to review disease education and support services. Earlier visits may be required because of a change in disease status, symptoms of depression, or medication monitoring (response and side effects).

Twenty consecutive outpatients diagnosed with MCI and for whom 2 year follow-up data was available or who had reached the study endpoint (Progression to AD) were assessed. Patients who met the following criteria were diagnosed with MCI:

- Age greater than 50 years
- Subjective and corroborated complaints of memory loss

- Objective cognitive impairment
- Gradual onset of cognitive impairment
- Cognitive impairment not due to reversible causes
- Not demented

The study endpoint (diagnosis of AD) was based on the NINCDS-ADRCS criteria[13].

Analysis

To determine whether there was a difference in baseline ADL scores between those who remained stable with MCI vs. those who progressed to AD in the subsequent 24 months, an independent samples t-test was done. An analysis was done on each ADL to determine whether differences in particular activities were more predictive of progression to AD.

The Lawton-Brody ADL scales (IADL & SCADL) were treated as ordinal scales for the purposes of this analysis, with a score of 1 reflecting no dependence, and higher scores reflecting increasing dependence. The range of possible scores for the L-B IADL is 8-30. The same approach was used for the L-B SCADL, providing a range of scores from 6 to 29, with higher scores reflecting greater dependence.

Results

Twenty outpatients (12 women) were used in this analysis. The average age of patients at diagnosis of MCI was 71.8 years (range 54-87). The majority of participants had at least 12 years of education. The Mini-Mental Status Examination (MMSE)[14] score at baseline was 27.3 (SD = 2.1). The mean baseline L-B IADL score was 11.6 (SD = 3.5) and baseline L-B SCADL score was 6.4 (SD = 0.6) (Table 1).

Eight of the participants (40%) had progressed to Alzheimer disease by the end of 24 months (20% mean annual progression rate).

Table 1. Mean baseline scale scores.

Measurement (Potential Scores)	Mean Baseline Total Score
MMSE (0-30)	27.3 (SD 2.1, range 23-30)
L-B IADL (8-30)	11.6 (SD 3.5, range 8-20)
L-B SCADL (6-29)	6.4 (SD 0.6, range 6-8)

There was no significant difference in age, gender, or education in those who progressed compared to those patients who remained stable. Total MMSE or L-B ADL scores at baseline did not predict who would develop Alzheimer disease in the subsequent 24 months (Table 2).

Examination of the subscale scores revealed that only one of the six IADL subscales was predictive of change with none of the SCADL contributing to the predictive value (Table 3 and Table 4, respectively). *Food Preparation* was the only subscale item at baseline that was significantly different between those who progressed to Alzheimer disease and those whose diagnosis remained stable at 24 months. This subscale item was independent of gender in this sample.

We examined the distribution of our sample by analyzing *Food Preparation* as a binary variable to predict progression to AD, with 'any impairment in Food Preparation' at baseline versus 'no impairment in *Food Preparation*' (Table 5). People with any impairment in *Food Preparation* at baseline were significantly more likely to progress to AD than those with no impairment (Odds Ratio = 8.33, Confidence Interval = 1.03 – 67.14).

Discussion

In this small study population of relatively high functioning adults with MCI, we found that a decreased capacity to prepare food at the time of diagnosis of MCI was significantly predictive of those who would be diagnosed with Alzheimer disease in the subsequent 24 months. The mean rate of progression was 20% per year, which is within the range of previous findings[2]. As would be expected, given the subtle nature of this disorder, we did not find that the baseline total MMSE score was useful in predicting who would progress to AD within 2 years.

Although *Handling Finances* was previously demonstrated to be predictive of AD[11], we did not replicate that finding in our population, however we did not address the question with the same level of sophistication

Table 2. Baseline characteristics by follow-up status.

Assessment Type	Patient status at Follow-up	Mean	SD
Age at Diagnosis	Progressed	72.42	5.4
	Stable	71.20	10.1
Mini-Mental State Exam	Progressed	27.0	1.8
	Stable	27.4	2.4
Self-Care ADL's Total Score	Progressed	6.5	0.5
	Stable	6.3	0.6
Instrumental ADL's Total Score	Progressed	13.1	3.6
	Stable	10.6	3.1

Table 3. Mean score of Instrumental Activities of Daily Living by follow-up status.

Instrumental Activity of Daily Living	Patient status at Follow-up	Mean	SD	p
Ability to Use Telephone	Progressed	1.4	0.5	0.38
	Stable	1.5	0.9	
Shopping	Progressed	1.9	0.9	0.42
	Stable	1.6	0.7	
Food preparation*	Progressed	2.0	1.1	0.02
	Stable	1.1	0.3	
Housekeeping	Progressed	1.8	1.0	0.11
	Stable	1.2	0.4	
Laundry	Progressed	1.0	0.0	0.46
	Stable	1.2	0.6	
Mode of Transportation	Progressed	2.1	1.4	0.24
	Stable	1.5	1.0	
Responsibility for Own Medications	Progressed	1.8	0.7	0.19
	Stable	1.3	0.7	
Ability to Handle Finances	Progressed	1.4	0.5	0.66
	Stable	1.3	0.5	

* $p < 0.05$

Table 4. Mean score of Basic Activities of Daily Living by follow-up status.

Basic Activity of Daily Living	Patient status at Follow-up	Mean	SD	p
Toileting	Progressed	1.0	0.0	0.22
	Stable	1.2	0.4	
Feeding	Progressed	1.1	0.4	0.13
	Stable	1.0	0.0	
Dressing	Progressed	1.0	0.0	*
	Stable	1.0	0.0	
Grooming	Progressed	1.3	0.5	0.09
	Stable	1.0	0.0	
Physical ambulation	Progressed	1.1	0.4	0.82
	Stable	1.1	0.3	
Bathing	Progressed	1.0	0.0	*
	Stable	1.0	0.0	

*Analysis not performed because there was no variability

Table 5. Patient impairment in food preparation by follow-up status.

	Progressed by 24 months	Stable at 24 months
Any Impairment in Food Preparation	**5**	**2**
No Impairment in Food Preparation	**3**	**10**

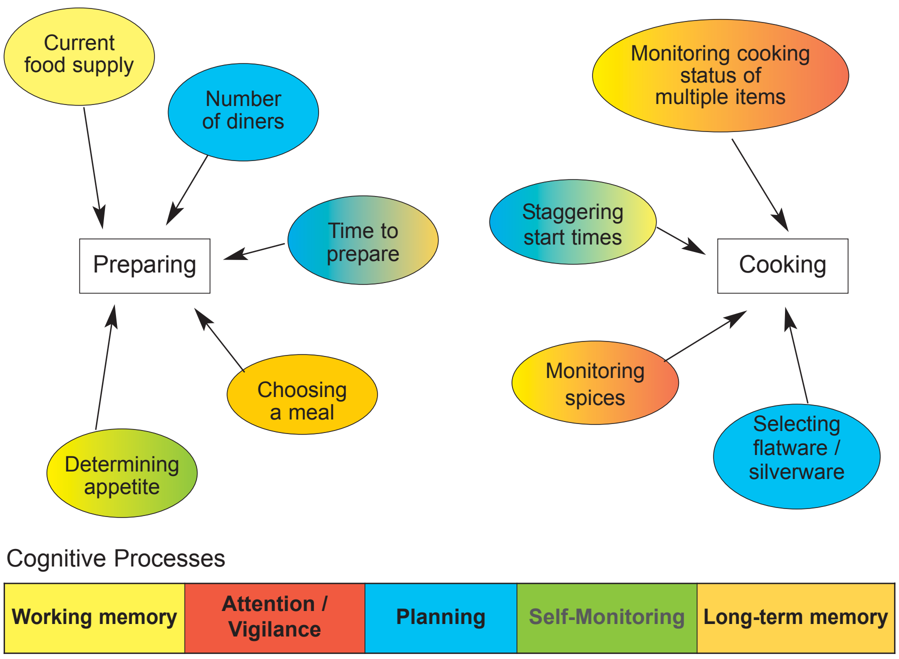
as did the previous authors. We suspect a strong gender bias for that variable in our clinic population, and due to the limited number of men in our sample, this study may not have had a sufficiently large sample size to demonstrate the same effect.

The significant finding of this study possesses face validity and is easily approached with patients and caregivers in a clinic setting. We hypothesized that the manifestation of functional deficits in MCI would affect a higher-order skill. *Food Preparation* fits well within this schema, as it incorporates many higher levels of cognitive functions (attention, vigilance, planning, and organization). Figure 1 graphically demonstrates which stimuli and processes are involved in planning and preparing a meal.

Another benefit to using *Food Preparation* is that it is something that the caregiver (usually a spouse or adult child) is very familiar with and has an internal previous benchmark of optimal performance to which they can compare the patient's current level of functioning. Their kitchen is also a constant environment, which helps reduce the amount of confounding variables. Further, while gender influences may affect the degree of involvement or level of sophistication generally involved in *Food Preparation*, we did not find that gender was a significant variable in predicting progression. We found that everyone in this population prepared at least a breakfast or lunch item for themselves. It is likely that those individuals, who do not have very advanced meal preparation skills, also do not spend as much time doing these activities, thereby reducing their practice effect or familiarity with the tasks. This lack of practice would cause even a slight decrease in function to be noticeable to family members.

While this exploratory study needs to be replicated in a larger sample to demonstrate the reliability of these findings, it is encouraging that readily measurable functional changes, tailored to an individual's

Figure 1. Cognitive processes associated with food preparation



situation, may be predictive of progression from Mild Cognitive Impairment to Alzheimer disease. A limitation to this method of assessment is its reliance on an informant. There are certainly situations where patients are socially isolated and would not have someone to corroborate their history or know their optimal level of function in an activity. Further research may find other tests or markers of executive function that may be reliable prognostic markers of progressive MCI.

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Efficacy of Rivastigmine in Patients with Severe Alzheimer's Disease: A Double-Blind, Randomized Pilot Study

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Abstract

Cholinesterase inhibitors are widely used for the treatment of mild-to-moderate Alzheimer's disease (AD), but there are limited data on the use of these agents in more severe disease. This multicentre, randomized, double-blind, placebo-controlled, 26-week, Spanish pilot study aimed to evaluate the safety and efficacy of rivastigmine in patients with more advanced AD. Patients were aged 55 years, and had a diagnosis of AD that was graded moderately severe-to-severe according to baseline Mini-Mental State Examination scores of 5–12 and Global Deterioration Scale stages of 5–6. Patients received placebo or rivastigmine 3–12 mg/day. A number of efficacy measures were used, including measures of cognitive performance, activities of daily living, neuropsychiatric symptoms and global impression of change. Adverse events were monitored throughout the study. In total, 218 were randomized to treatment (109 per treatment group). Rivastigmine provided statistically significant improvements, compared with placebo, on measures of cognitive performance and global impression of change. Improvements were also reported on activities of daily living and neuropsychiatric measures of efficacy (not statistically significant). Adverse events were reported by 88.1% of rivastigmine- and 68.8% of placebo-treated patients; 9.2% of the rivastigmine group and 4.6% of the placebo group withdrew due to adverse events. In conclusion, the findings of this small, but well-controlled, pilot study suggest that patients with moderately-severe-to-severe AD receiving rivastigmine treatment obtain significant benefits over placebo, according to classical AD outcome measures of cognition and global functioning. Rivastigmine did not appear to present additional safety concerns in this population. Further, larger studies of cholinesterase inhibitors in advanced AD would be worthwhile.

Keywords: *Rivastigmine, Severe Alzheimer's disease, Cognitive impairment, Efficacy, Safety, Tolerability*

Introduction

The cholinergic system has an important role in memory function and also in regulating regional cerebral blood flow, which can enhance cortical function[1]. Reduced cholinergic activity correlates with amyloidogenic metabolism and the progressive cognitive and memory impairment, and behavioural disturbances, that are characteristic of Alzheimer's disease (AD)[2,3]. This neurodegenerative disorder is characterized by a progressive loss of cholinergic neurons, and their cortical projections, from the nucleus basalis and associated basal forebrain areas[4-6].

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) appear to be simultaneously active in the synaptic hydrolysis of acetylcholine (ACh), terminating its neurotransmitter action, and co-regulating levels of ACh to facilitate cholinergic neurotransmission[7]. Levels of AChE in the brain decrease as AD progresses, whilst BuChE levels increase by 30-60% in the AD brain, probably due to an increase in the number of BuChE-positive glia[8,9]. In AD, glial BuChE-to-AChE ratios also increase in brain regions with a high susceptibility to AD pathology, such as the entorhinal and inferotemporal cortices[10]. Since glial BuChE may co-regulate ACh, analagous to the role of glia in

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glutaminergic neurotransmission[11], elevated levels of BuChE in advanced AD may exacerbate the reductions in cholinergic neurotransmission by further decreasing ACh levels. Furthermore, BuChE activity in the temporal cortex and variant BuChE alleles have been correlated with the rate of cognitive decline in patients with dementia[12,13]. Although the role of BuChE in AD pathogenesis remains to be determined, these findings suggest that it may be an important target, in addition to AChE, in the treatment of AD, particularly in those patients with more advanced disease.

Cholinesterase inhibitors (ChEIs) reduce the breakdown of ACh and are approved for the treatment of mild-to-moderate dementia of the Alzheimer type[14,15]. The most commonly-used ChEIs are donepezil, rivastigmine and galantamine. Rivastigmine is a slowly reversible inhibitor of both AChE and BuChE, whereas donepezil and galantamine are specific and rapidly reversible inhibitors of AChE[16]. According to the National Institute of Clinical Excellence (NICE) in the UK, there are insufficient data to support the use of these agents in patients with more severe disease (i.e. Mini-Mental State Examination [MMSE] scores ≤12[15].

Several trials have demonstrated that rivastigmine is effective in mild to moderately-severe AD patients[17-19]. A recent meta-analysis of these trials indicated that rivastigmine provided cognitive and behavioural benefits to AD patients with more advanced disease (MMSE scores ≤ 12)[20]. In addition, there is evidence from other trials to suggest that patients with more advanced AD might respond to ChEI treatment more strongly than those with mild symptoms. At the time the present study was designed, the consensus was that patients with severe AD would have such extensive neurodegeneration that they would not experience the symptomatic benefits from ChEI therapy seen in by those with mild-to-moderate AD. Thus, it was not considered appropriate to perform a large, fully powered, ChEI trial on this patient population. Therefore, we performed a pilot study, which evaluated the cognitive, behavioural, and global efficacy of rivastigmine in patients with severe AD, to determine whether such patients could still respond to ChEI therapy.

Methods

Patients

Consenting men or women aged 55 years, diagnosed as having dementia of the Alzheimer type (DSM-IV criteria) and probable AD (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association or NINCDS-ADRDA criteria) graded moderately severe-to-severe (MMSE scores 5–12; GDS stage 5–6), with a modified Hachinski scale score 4[21], were recruited. Patients included in this study also had daily contact with a caregiver who agreed to assist with study evaluations.

Exclusion criteria included: an exaggerated pharmacologic sensitivity or hypersensitivity to rivastigmine or other cholinergic-like drugs (e.g. tacrine, physostigmine); a history of drug abuse; diagnosis of any severe advanced medical disease (including primary neurological and/or psychiatric diseases) or any disorder considered by the investigator as likely to impair clinical evaluations or affect the mental state of the patient; severe and unstable cardiovascular disease; ongoing sinus node dysfunction or conduction disturbance (sinoatrial block, second or third degree atrioventricular block); institutionalization; ingestion of other ChEI inhibitors, investigational drugs or drugs known to result in toxicity to the main organ systems, within 4 weeks of study-entry.

Study design

This multicentre, randomized, double-blind, placebo-controlled, parallel-group, 26-week pilot study of rivastigmine was conducted at 21 Neurology, Psychiatry and Geriatric Units in Spain. Eligible patients, identified at an initial screening visit, were allocated a randomization number at a second visit, according to a previously prepared computer-generated randomization schedule. Randomization was performed by a CRO, Clinical Data Care AB (CDC) in Lund, Sweden. The randomization scheme was reviewed by the Quality Management Biostatistics Group in the Novartis Statistics and Medical Information Processing Department, in Basel, Switzerland. Blocking procedures were used for the randomization (block size: 4).

Study medication (rivastigmine or placebo) was issued at each centre and patients received treatment for 26 weeks. The medical monitors, centre personnel, patients and caregivers were blinded to treatment. Randomization data were not available until after the study had been completed and the database locked. Rivastigmine and placebo hard capsules were identical in appearance during this double-blind phase. Patients initially received dose level 1 (rivastigmine 1.5 mg b.i.d. or placebo b.i.d.) for 4 weeks and, if this dosage was well-tolerated, they received dose level 2 (rivastigmine 3 mg b.i.d. or placebo b.i.d.) for a further 4 weeks. Providing there were no tolerability problems, patients were up-titrated to dose levels 3 (rivastigmine 4.5 mg b.i.d. or placebo b.i.d.) and 4 (rivastigmine 6 mg b.i.d. or placebo b.i.d.) at 4-week intervals. In the case of tolerability problems, the dose could be decreased to the previous well-tolerated dose level. Written informed consent was received from all patients (or from their guardians/legal representatives if not mentally competent) at the baseline screening visit, prior to enrolment. This study received ethical committee approval and was conducted in accordance with good clinical practice and the Declaration of Helsinki.

Efficacy Measures

Cognitive performance and behavioural and psychiatric symptoms were evaluated in this study, at baseline (screening visit) and after 26 weeks of treatment using the Severe Impairment Battery (SIB)[22] and the 10-item Neuro-psychiatric Inventory (NPI-10)[23] as primary efficacy measures. The SIB was developed to grade cognitive performance in seriously handicapped patients unable to complete conventional neuropsychological tests. SIB scores range from 0 (worse) to 100 (better)[22]. The NPI is a structured caregiver interview that evaluates behaviour and other psychiatric symptoms in demented patients and differentiates between the frequency and severity of psychiatric symptoms[22]. The NPI is measured on a scale from 0–144 with higher scores representing increasing levels of behavioural/ psychiatric disturbance. The NPI-10 covers 10 domains: delusions, hallucinations, agitation/aggression, depression/dysphoria, anxiety, elation/euphoria, apathy/ indifference, disinhibition, irritability/lability, and aberrant motor behaviour[22]. When possible, efficacy assessments were performed by the same person at each centre to ensure reliability.

Secondary outcome measures included the MMSE[24], the Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC)[25], the Global Deterioration Scale (GDS)[26], the Blessed Scale[27], the Alzheimer's Disease Cooperative Study–Activities of Daily Living Inventory (ADCS-ADL)[28], and the 4-item NPI (NPI-4)[22]. The MMSE comprises a series of questions designed to assess orientation, attention, immediate and short-term recall, language, and the ability to follow simple verbal and written commands, and is graded on a 30-point scale[24]. MMSE scores of 5–12 indicate moderately severe-to-severe cognitive impairment. The ADCS-CGIC is a 7-point scale (where 1 = marked improvement and 7 = marked deterioration) eliciting the clinical information necessary from both caregiver and patient to measure clinical global impression of change compared with the baseline visit[25]. The GDS is another 7-point scale that assesses the clinical staging of the natural history of AD, scored from 1 (mild) to 7 (severe)[26].

The Blessed scale is a multidimensional performance scale in three main sections, each divided into subsections scored 0, 0.5 or 1, to a total of between 0 and 28[27]. The ADCS-ADL rating scale is a 23-item assessment of ADLs that is scored from 0 (greatest impairment) to 78[28]. The NPI-4 covers four behavioural domains: delusions, hallucinations, depression and apathy[22].

Safety and tolerability

General clinical safety was assessed by monitoring adverse events, changes in clinical laboratory variables (haematology; biochemistry, including liver function tests [e.g. alanine aminotransferase; aspartate aminotransferase]), and vital signs, body weight, electrocardiograms, and physical examinations. Tolerability was assessed by recording all reported side effects.

Statistical analyses

The global NPI-10 score was utilized to determine sample size using Nquery software (Windows 95). Ninety-two patients per treatment group (184 in total) were considered sufficient to achieve 80% power and 5% significance in a two-sided test, assuming a standard deviation of 12 and a 5-point difference between treatment group means. Therefore, assuming a 15% drop-out rate, 109 patients (218 in total) needed to be included in each treatment group to guarantee the required total of 184 patients. Efficacy analyses were based on the intention-to-treat (ITT)-last observation carried forward (LOCF) cohort, which comprised all randomized patients who had received at least one dose of study medication and had at least a baseline and a post-baseline efficacy assessment for one of the primary efficacy variables. Changes in cognitive and/or behavioural symptoms from baseline to 26 weeks between treatment groups were determined by analysis of covariance or ANCOVA (SIB scores; NPI-10 scores) adjusting for centre, treatment, baseline value and, if statistically significant, treatment per baseline value. Associated 95% confidence intervals (CI) along with *p*-values were calculated for the differences between the two treatment groups. The Wilcoxon rank sum test was used to evaluate changes between treatment groups in mean MMSE, ADCS-CGIC, GDS, NPI-4, and ADCS-ADL scores, from baseline to 26 weeks.

Results

Disposition and patient demographics

The first patient was recruited in September 2000 and the last patient completed the study in October 2002. Of 238 patients screened, 218 (109 per treatment group) were randomized to receive rivastigmine 6–12 mg/day or placebo. In total, 91 (83.5%) patients receiving rivastigmine and 96 (88.1%) in the placebo treatment group completed the study, with the remainder discontinuing after receiving at least one dose of study medication (Figure 1). Five patients in the rivastigmine group and three in the placebo group received no post-treatment SIB or NPI assessments and were therefore excluded from the efficacy analyses. The ITT population comprised 104 patients in the rivastigmine group and 106 in the placebo group.

The treatment groups had similar baseline demographics and patient characteristics (Table 1). Overall, similar proportions of

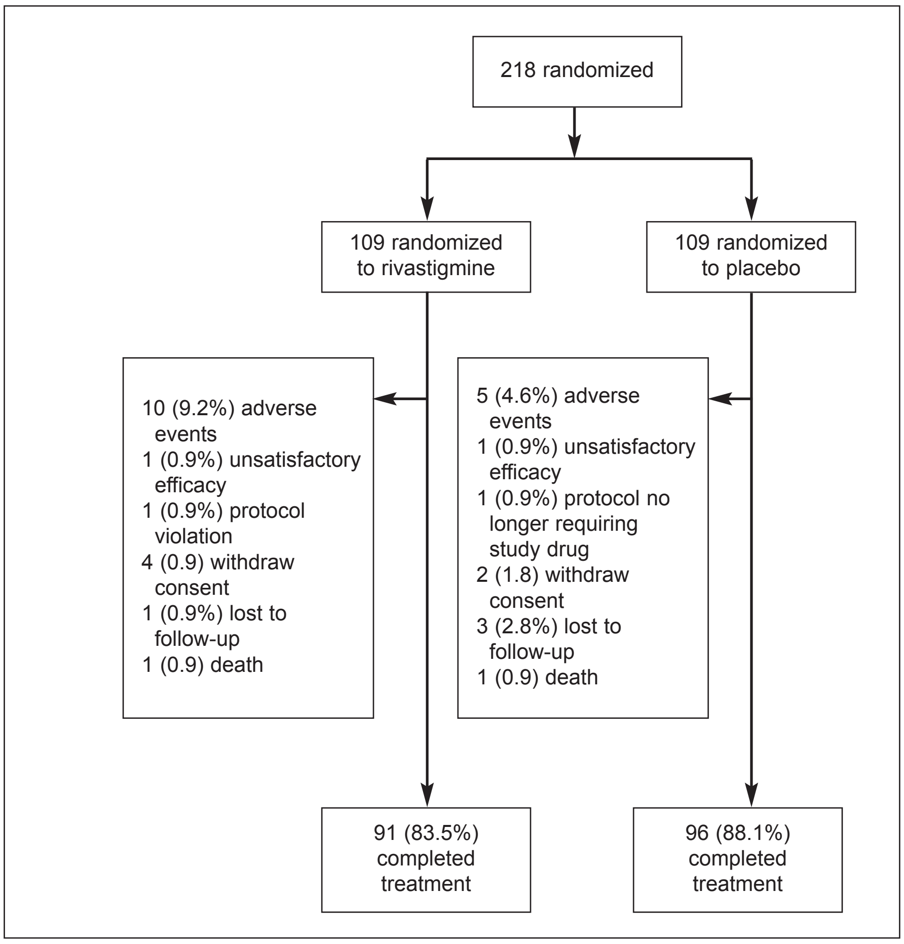

Figure 1. Patient disposition.

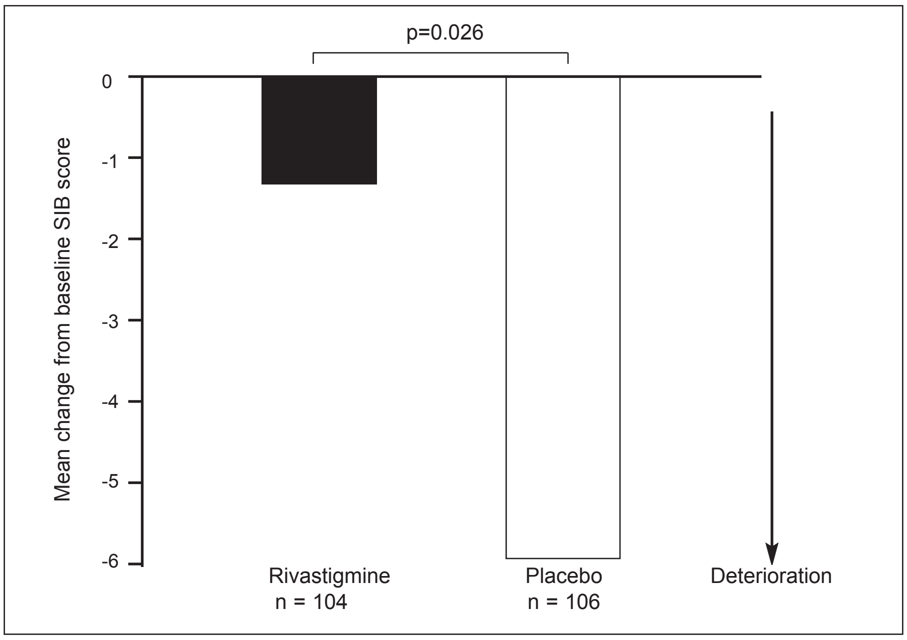

Figure 2. Mean changes from baseline scores on the Severe Impairment Battery (SIB) scale following 26 weeks of treatment with rivastigmine or placebo, in patients with moderate-to-severe Alzheimer's disease.

patients within each group were taking concomitant antipsychotic and anxiolytic drugs at baseline and/or during the study. However, the proportion of patients receiving antidepressants or hypnotics at baseline was approximately double that in the rivastigmine group, relative to the placebo group (Table 1). The mean (SD) duration of exposure to study medication was 168.2 (47.2) days for patients receiving rivastigmine and 172.9 (39.0) for those receiving placebo treatment. The mean (SD) dose received in the rivastigmine group at the end of the study was 9.8 (2.8) mg/day.

Cognitive performance

The decline in mean SIB score from baseline to the end of the 26-week study period was significantly greater in the placebo group than in the rivastigmine group (treatment difference 4.0 points, $p = 0.026$) (Figure 2). The adjusted difference between the means (95% CI) was 3.83 (0.46, 7.21).

At study endpoint, mean changes in MMSE scores following rivastigmine treatment improved by 0.6 from baseline values compared with a decline of 1.0 in the placebo group. This represented a statistically significant treatment difference of 1.6 ($p < 0.001$) (Figure 3).

Behavioural symptoms

Patients receiving rivastigmine treatment showed an improvement in mean NPI-10 score of 0.1 points over 26 weeks, compared with a worsening of 1.7 points in those receiving placebo (Table 2). The adjusted difference between the means was 0.92 points ($p = 0.64$).

The NPI-4 also showed a trend for behavioural benefits with rivastigmine compared with placebo. At 26 weeks, rivastigmine-treated patients showed an improvement of 0.3 points, compared with a worsening of 2.0 points in those

Table 1. Baseline demographics and patients characteristics (all patients randomized, n=218).

Baseline demographic/characteristic:	Rivastigmine 6-12 mg daily (n = 109)	Placebo (n = 109)
Mean age, years (range)	78.0 (60-89)	77.2 (57-92)
Gender (% women : men)	78.0 : 22.0	76.1 : 23.9
Mean (SD) MMSE score	9.0 (2.2)	8.7 (2.2)
Patients receiving antipsychotics, n (%)	27 (24.8)	23 (21.1)
Patients receiving anxiolytics, n (%)	14 (12.8)	15 (13.8)
Patients receiving antidepressants, n (%)	15 (13.8)	7 (6.4)
Patients receiving hypnotics, n (%)	10 (9.2)	6 (5.5)

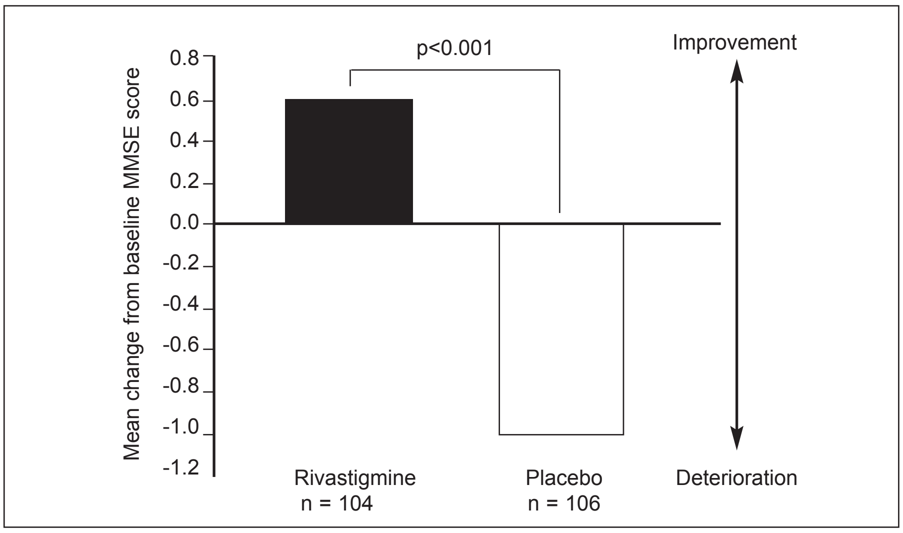

Figure 3. Mean changes in MMSE scores from baseline values after 26 weeks of treatment with rivastigmine or placebo, in patients with moderate-to-severe Alzheimer's disease.

At baseline, both groups had a mean GDS score of 5.5, representing severe AD. At the end of the study, placebo-treated patients had deteriorated to a mean score of 5.8 (0.3-point change from baseline), while those in the rivastigmine group had a mean score of 5.6 (0.1-point change from baseline). However, the between-treatment difference on the GDS did not reach statistical significance (p=0.065). The between- treatment difference on the Blessed scale was also non-significant (p=0.63).

Activities of daily living

On average, patients receiving rivastigmine deteriorated by 3.9 points on the ADCS-ADL, compared with 5.6 points (adjusted mean difference: 1.7 points) for those receiving placebo (p = 0.23).

Safety and tolerability

The overall adverse event incidences were 88.1% for the rivastigmine group and 68.8% for the placebo group (Table 3). Overall, 9.2% of patients in the rivastigmine group and 4.6% in the placebo group withdrew due to adverse events, the majority of which were mild-to-moderate in severity. There were two deaths during the study period, 1 in each treatment group. The death in the rivastigmine group

receiving placebo. The adjusted difference between the means was 1.56 points (p = 0.11).

Global impression of change

The ADCS-CGIC results also demonstrated the significant global benefits of rivastigmine, with the symptoms of 22.1% (n=23) of treated patients improving compared with improvements in only 9.6% (n=10) of those receiving placebo. The global benefits of rivastigmine were significantly greater than placebo at the end of the 26-week treatment period (p = 0.024).

Table 2. Behavioural efficacy as assessed by the NPI-10: prospective, placebo-controlled study (ITT population).

	Rivastigmine6-12 mg daily(n = 104)	Placebo(n = 106)
Baseline (week 0) mean NPI-10 score (± SD)	21.5 (15.3)	19.8 (17.1)
Final (week 26) mean NPI-10 score (± SD)	21.4 (17.2)	21.5 (17.5)
Mean change in NPI-10 score from baseline (± SD)*	- 0.1 (15.2)	1.7 (17.5)

*A negative mean change from baseline represents an improvement in behavioural symptoms. There were no significant differences between treatment groups.
NPI-10: 10-item Neuropsychiatric Inventory

Table 3. Adverse events incidences.

Adverse events*	Rivastigmine 6-12 mg daily (n = 109)	Placebo (n = 109)
Total adverse events, n (%)	96 (88.1)	75 (68.8)
Total gastrointestinal adverse events, n (%)	54 (49.5)	21 (19.3)
Total psychiatric adverse events, n (%)	32 (29.4)	20 (18.3)
Total nervous system adverse events, n (%)	27 (24.8)	18 (16.5)
Most common adverse events (³ 10%)		
Nausea, n (%)	28 (25.7)	2 (1.8)
Vomiting, n (%)	23 (21.1)	4 (3.7)
Anorexia, n (%)	11 (10.1)	2 (1.8)

*Patients experiencing multiple adverse events within any of the listed categories were counted only once

was suspected to be treatment-related. Seven non-fatal serious adverse events were reported in 6 patients receiving rivastigmine (moderate abdominal pain, a mild transient ischemic attack, a nasal cavity cancer, a myocardial infarction, an abdominal strangulated hernia, face crushing, and syncope). Two of these serious adverse events (face crushing and syncope) were suspected to be treatment-related by the study investigator. A further 9 non-fatal serious adverse events were reported in 9 patients receiving placebo. The majority of adverse events were gastrointestinal, nervous system or psychiatric disorders (Table 3). The most common adverse events included nausea, vomiting, dizziness, insomnia, diarrhoea and anorexia. The incidences of nausea, vomiting and anorexia were greater for rivastigmine than placebo treatment (Table 3).

There were no clinically relevant changes in mean systolic and diastolic blood pressures or heart rate throughout the study. There was one death due to myocardial infarction in each treatment group. At week 26, two patients (1.8%) receiving rivastigmine and two patients (1.8%) in the placebo group had clinically significant abnormalities upon ECG evaluation. However, the clinically significant abnormality was already present at baseline for one of the patients in the rivastigmine group. In addition, there was a low incidence of newly occurring clinically significant laboratory test abnormalities at the end of the study in each treatment group. Four (4.1%) patients in the rivastigmine group experienced abnormally high blood glucose levels compared with five (4.8%) in the placebo group. Abnormally low haematocrits were observed in two (1.9%) patients receiving rivastigmine compared with five (4.7%) patients receiving placebo. Abnormal elevations in liver function markers (AST) were only observed in one (0.9%) patient who received rivastigmine.

Discussion

This was a small, though well controlled, prospective pilot study in which recommended therapeutic doses of rivastigmine (6–12 mg daily) provided significant cognitive and global improvements over placebo in patients with moderately severe to severe disease. Rivastigmine had a stabilizing effect, with treated patients experiencing significantly less cognitive decline from baseline levels over a 26-week treatment period, compared with those receiving placebo. Disease staging, as assessed by the GDS, was relatively stable over the course of the study. These findings support the use of ChEIs in patients with advanced AD. Although patients receiving rivastigmine also showed improvements in their behavioural symptoms after 26 weeks, while the behavioural symptoms of those receiving placebo worsened, these differences were not statistically significant in this small, 'close-to-clinical practice' pilot study. Similarly, although patients receiving rivastigmine experienced less impairment of their ADLs than those receiving placebo, this difference was not statistically significant in this small pilot study.

The present cognitive findings are in accordance with evidence in the form of case studies, post-hoc analyses, and controlled clinical trials, which support the cognitive efficacy of ChEIs in patients with moderate-to-severe and severe AD[29-33]. In a post-hoc analysis, galantamine efficacy data for an 'advanced moderate' AD subset was derived from two long-term studies and was compared with matched placebo from a different historical study. In total, 51% of patients receiving galantamine maintained or improved their cognitive performance over the 52-week treatment period compared with only 13% in the historical placebo group (originally from another study)[32]. Donepezil has also demonstrated cognitive efficacy in clinical trials in non-institutionalized AD patients with moderate to moderately-severe symptoms (MMSE scores 5–12)[33], though not in Nursing Home patients[30].

The behavioural efficacy of ChEIs, including rivastigmine, has been demonstrated in several clinical trials of patients with more severe AD[20,33]. Similarly, ChEIs including rivastigmine have shown benefits on ADLs in several AD trials[34]. The improvements in behavioural symptoms and the reduced impairment of ADLs with rivastigmine relative to placebo treatment in the present small, pilot study, although not significant, are consistent with these published observations. However, the non-significant findings of this 'close-to-clinical practice' study indicate that, in order to attain statistical significance, the sample size needs to be higher and the use and change of co-medication needs to be more restrictive. For instance, the larger proportion of AD patients within the rivastigmine group receiving concomitant antidepressants and/or hypnotics at baseline, which was approximately twice that in the placebo group, may have affected the NPI-10 results.

This study was performed in a Spanish population. We recently performed a epidemiological study in a population cohort aged at least 75 years (n = 1990), in which it was shown that incidence rates per 1,000 person-years at risk were 23.2 (95% CI = 19.1-27.3) for dementia, and specifically 10.8 (95% CI = 7.8-13.7) for AD[35]. These incidence rates represent a considerable burden on the Spanish healthcare system, although the provision of effective treatment may have the potential to reduce many indirect costs of the disease. In particular, in a separate study, we showed that the cost per AD patient per month increases dramatically with disease severity: 419.3 Euros for patients with MMSE scores above 19, 641.9 Euros for those with MMSE scores of 11 to 19, and 1150.6 Euros for those with MMSE scores of less than 11[36]. The patients in the current study had MMSE scores of less than 12, and therefore fall into the most expensive bracket of patients. Effective treatment of these patients may have considerable societal benefits, and is worthy of further investigation.

It is important to note that many of the studies and analyses evaluating the efficacy of the ChEIs in patients with more severe AD have been performed on non-institutionalized subjects and may not, therefore, reflect the Nursing Home population in general. However, there is some preliminary evidence from open-label studies to suggest that rivastigmine improves cognitive and behavioural symptoms in Nursing Home patients with moderate-to-severe AD[37,38], including an associated reduction in their psychotropic drug-use[39]. However, double-blind studies will be required to confirm the efficacy of ChEIs, such as rivastigmine in Nursing Home patients with more severe AD.

The efficacy of rivastigmine in AD patients with more severe disease in studies to date may be related to its ability to inhibit BuChE in addition to AChE, and its selectivity for brain regions (e.g. hippocampus and cortex) in which cholinergic deficits are most pronounced in AD[40,41]. These regions with more advanced AD pathology are also those regions which show the most substantial increases in BuChE activity (> 100 Aβ deposits per mm^3 of brain). Furthermore, patients with severe AD may have a greater potential for improvement with ChEI therapy on the basis that they have a larger cholinergic deficit than patients with milder symptoms[42]. However, the role of BuChE in the aetiology of AD and its involvement in the efficacy of rivastigmine remain to be clarified.

In the present small, prospective placebo-controlled pilot study, rivastigmine was relatively well-tolerated by patients with moderately severe to severe AD, with gastrointestinal events being the most commonly reported adverse events. The incidences of withdrawal due to adverse events for rivastigmine-treated patients were approximately twice the placebo rate. Gastrointestinal adverse events are common with ChEI therapy and are mostly reported during the dose-escalation phase, particularly in rapid forced titration studies. The present pilot study, like more recent rivastigmine clinical trials and in routine clinical practice, used a slow, flexible dose escalation schedule, with a minimum 4-week interval between dose increases, which has been shown to markedly reduce the incidence of gastrointestinal events such as nausea and vomiting[43]. This likely explains the observed low discontinuation rate for adverse events (9.2%) and low overall and individual incidences of GI adverse events associated with rivastigmine in this study (Tables 1 and 3).

It has been previously reported by Burns *et al*[20] that the frequency and type of adverse events are similar for patients with moderately severe AD and those with mild symptoms. Burns *et al*[20] also found that the relative risk of withdrawal due to adverse events calculated for patients with moderately severe AD receiving rivastigmine *versus* those receiving placebo (2.0 [95% CI: 0.9–4.6]) was lower than for patients with mild AD (3.6 [95% CI: 2.4–5.4; for patients with MMSE scores 22–26]). This suggested that the relative risk of withdrawal is related to the level of baseline cognitive impairment and that there are no additional tolerability concerns associated with rivastigmine treatment for patients with more severe AD relative to those with earlier stage AD.

NICE recommendations do not support the use of ChEIs in patients with MMSE scores below 12. However, the present findings and those of other clinical trials support the efficacy of rivastigmine and other ChEIs[29-33] in patients with more severe AD, suggesting that an arbitrary cut-off point of 12 points on the MMSE may not be an appropriate basis for denying patients ChEI treatment. The present prospective placebo-controlled study evaluated patients with MMSE values as low as 5. Hence, while the current NICE limit of 12 could prevent patients with more severe symptoms from benefiting from ChEI therapy, more controlled trials will be needed to determine the most appropriate, lower ChEI eligibility threshold.

The findings of this small, but well-controlled pilot study, suggest that patients with moderately severe to severe AD receiving rivastigmine treatment obtain significant benefits over placebo, according to the classical AD outcome measures of cognition and global functioning. Rivastigmine had a stabilizing effect delaying the deterioration of cognitive symptoms of patients with more severe AD in comparison with those receiving placebo. The non-significant trend towards improved behavioural symptoms and reduced impairment of ADLs with rivastigmine relative to placebo emphasizes the need for future 'close-to-clinical practice' AD trials to be larger and fully powered.

The following were principal investigators in this study:
L. Agüera (Madrid); M. Aguilar (Barcelona); E. Balaguer (Barcelona); M. Berthier (Sevilla); R. Blesa (Barcelona); M. Boada (Barcelona) M. Franco (Zamora); A. Frank (Madrid); P. Gil (Madrid); L.M. Iriarte (Sevilla); J.M. Lainez (Valencia); S. López-Pousa (Girona) Lead investigator; R. Mateos (La Coruña); J. Matias-Guiu (Alicante); E. Muñoz (Tarragona); J. Peña (Barcelona); A. Robles (La Coruña); I. Sánchez (Toledo); C. Sevilla (Madrid); J. Vilchez (Valencia).

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The Histamine H2 Receptor Antagonist Cimetidine Activates Microglia in APP+PS1 Transgenic Mice

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Abstract

Epidemiological studies report an inverse association between use of histamine H2 receptor antagonists and Alzheimer's disease. APP+PS1 transgenic mice deposit amyloid and develop memory deficits in a manner resembling that in Alzheimer's disease. We therefore fed a diet containing the H2 receptor antagonist cimetidine to transgenic mice and nontransgenic littermates between 3 and 15 months of age. Cimetidine did not modify the memory deficits found in the transgenic animals, but produced a slight reduction in Y maze activity in both transgenic and normal littermate animals. Cimetidine also failed to modify the deposition of amyloid in transgenic animals, as measured by Aß immunohistochemistry, Congo red staining or Thioflavine S staining, but significantly increased the microglial activation associated with amyloid deposits, as evaluated with CD45 and CD11b immunohistochemistry. No such increases were found in nontransgenic mice treated with cimetidine. We conclude that, if histamine H2 receptor antagonists are to offer effective prophylaxis against the development of Alzheimer's disease, the mechanism for this effect is independent of amyloid deposition.

Keywords: *Cimetidine, amyloid, microglia, H2-receptor, APP transgenic mouse*

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by senile plaques that primarily consist of complexes of the Aß protein forming fibrillar aggregates[1]. Loss of synapses, intracellular neurofibrillary tangles, dystrophic neurites and neuronal cell death are additional histopathological elements of AD[2]. These Aß deposits appear to provoke an inflammatory response, possibly by directly activating the complement pathway[3]. Histological evidence of the inflammatory response consists of activated microglia that are associated with the senile plaques in AD.

Epidemiological studies[4,5], have reported an inverse association between the use of histamine H2-receptor antagonists and the risk for AD. Cimetidine, a non-thiourea H2–receptor antagonist, is a drug used widely used for treatment of gastric hypersecretion and duodenal pathologies[6]. Cimetidine can penetrate the blood-brain barrier[7] and can be measured in both the brain (Kendall et al 1980, Smith et al 1980) and the spinal fluid[8].

The mechanisms by which H2 blockers might protect from AD are uncertain. One possible action is a reduction in the rate at which Aß deposits are formed in the CNS. The APP+PS1 transgenic mouse is a well characterized model of Aß deposition[10]. As they age, these mice develop amyloid plaques, dystrophic neurites, microglial activation and memory deficits[10-12]. Here we show that treatment of doubly transgenic APP+PS1 mice with cimetidine for

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12 months via drinking water results in a significantly increased level of microglial activation without changes in amyloid deposition.

Methods

Animal treatments

Transgenic Tg2576 APP mice[13] were bred with line 5.1 PS1 mice[14] several years ago to establish a doubly transgenic APP+PS1 mouse model of amyloid deposition[15]. We used such APP+PS1 doubly transgenic mice and nontransgenic littermates from two birth cohorts. Mice were 3 months of age at the initiation of the study. Animals were group housed and provided food and water *ad libitum*. Mice received vehicle (deionized water) or cimetidine (cimetidine hydrochloride U.S.P. oral solution 60 mg/ml, TEVA Pharmaceuticals, Sellersville, PA) via drinking water for a period of 12 months. Water consumption was unaffected by the presence of cimetidine and bottles were changed twice weekly. Cimetidine was started at a dose approximating 5 mg/kg for the first week, and the dose was increased by 5 mg/kg every week until reaching a dose maximum approximating 40 mg/kg for females and 50 mg/kg for males (to accommodate known gender differences in cimetidine metabolism). All the mice were weighed twice weekly initially to monitor drug effects, and then weekly when stabilized on the drug. No differences in body weight were found between the vehicle and cimetidine treated groups. At 14 months of age mice were tested for the behavioral effects of cimetidine treatment and then sacrificed at 15 months after the completion of behavioral testing. Although we used group assignment intended to achieve approximately 9-10 mice in each genotype/treatment group, random variation and attrition resulted in final sample sizes of 7 for non-transgenic vehicle treated mice, 7 for non-transgenic cimetidine treated mice, 10 for transgenic vehicle treated mice and 5 for transgenic cimetidine treated mice.

Behavioral testing

There were two tests of motor coordination (balance beam and coat hanger test), the Y maze test of short-term memory and the radial arm water maze test of spatial navigation and memory. Detailed procedures describing these tasks have been published by us previously[11,12,16,17]. The coat hanger and balance beam tasks measured the time that mice remained suspended from a wire coat hanger (many mice shinny along the coat hanger to the corner and right themselves) or when placed upon a narrow wooden beam. These tests were repeated three times for each mouse and the times suspended were averaged to produce a single value for each mouse.

The Y maze was a three armed maze with equal angles between all arms. Mice were initially placed inside one arm and the sequence and number of arm entries was then recorded for each mouse over an 8 minute period. The percentage of triads in which all three arms were represented (ABC, CAB or BCA but not BAB) were recorded as an alternation to estimate short-term memory (mice will presumably be motivated to explore the more novel arms not visited on the last two choices). The total number of possible alternations is the number of arm entries minus two. In the radial arm water maze, a 6 arm maze was placed within a pool to produce swim alleys. At the end of one alley was a platform submerged just beneath the water. Each day a mouse attempted to learn the platform location for that day over 5 trials of 60 seconds each (4 consecutive trials and a 5th delayed by 30 minutes). Each trial started from a different arm (making this a spatial memory task requiring use of extra-maze cues) and each day the platform location was in a different arm. The number of incorrect arm entries (errors) was measured each day. Mice were trained on the task for 10 days, with stable performance emerging in the nontransgenic mice by the end of the testing period. Rather than averaging the last three days of testing, only days 8 and 10 were averaged because of disruption by unscheduled construction noise near the animal testing room part way through day 9 testing (day 9 data was discarded).

Histopathology

On the day of sacrifice the mice were overdosed with 100 mg/kg pentobarbital (Nembutal sodium solution, Abbott Laboratories, North Chicago IL) and perfused intracardially with 25 ml of 0.9% sodium chloride. Brains were rapidly removed and immersion fixed in freshly prepared 4% paraformaldehyde buffered with phosphate to pH = 7.4. After a 24 hour postfix, the brains were then incubated sequentially in 10%, 20%, and 30% sucrose (24 hours each) to cyroprotect them. Horizontal sections of 25 µm thickness were then collected using a sliding microtome and stored at 4°C in Dulbecco's phosphate buffered saline with sodium azide to prevent microbial growth. Eight sections spaced 600 µm apart were selected and stained using free-floating immunohistochemistry methods for activated microglia using CD45 (Serotec, Raleigh, NC, 1:3000) and CD11b (Serotec, Raleigh NC, 1: 3000), and for Aß (polyclonal rabbit antibody from Paul Gottschall, USF, Tampa, FL, USA). For immunostaining, some sections were omitted from the primary antibody to assess non-specific immunohistochemical reactions. Other sections were mounted on slides and stained for Congo red or Thioflavine S using standard protocols.

The histochemical reaction product was measured using computer-assisted image analysis software (Image Pro Plus, MediaCybernetics, Silver Spring, MD) in frontal cortex and hippocampus on both sides of the brain. All sections from a specific region in a single mouse were averaged to represent the single value for that mouse. Data are expressed as percent area occupied by positive stain.

To assess possible treatment-related differences, the behavioral scores or histochemical measurements for each subject were analyzed by ANOVA using Stat View software version 5.0.1 (SAS Institute Inc, Cary, NC) followed by Fischer' LSD means comparisons.

Results

Behavioral measures

There were very few effects of cimetidine on the behavioral outcomes of the study. In the Y-maze test, short term memory as measured by the percentage of alternations was significantly lower in the transgenic mice ($F_{1,26}$ = 4.6; $P < 0.05$), but there was no significant effect of cimetidine treatment, nor a significant interaction between cimetidine treatment and genotype (Figure 1A). In counting the number of arm entries in the Y maze, a measure of activity, there was a significant reduction with cimetidine treatment ($F_{1,26}$ = 4.5, $P < 0.05$), but no effect of genotype nor an interaction between genotype and cimetidine treatment (Figure 1B).

Mice also showed no significant overall effects of genotype or cimetidine in testing for spatial memory in the radial arm water maze task (Figure 2). Over the last two uninterrupted days of training there were no significant overall effects of genotype or cimetidine. However, when only vehicle treated mice were considered, the transgenic mice committed significantly more errors than nontransgenic mice on trial 4 ($F_{1,15}$= 4.8; $P < 0.05$) and trial 5 ($F_{1,15}$ = 6.9; $P < 0.02$) than nontransgenic mice. The absence of an effect of genotype in cimetidine treated mice is associated with slightly better performance in the transgenic and slightly worse performance in the nontransgenic mice. Thus, we draw no conclusions from the observation that cimetidine treatment eliminated the transgenic effect found in mice treated with vehicle.

We also tested mice on the coat hanger test and the balance beam, tasks on which the transgenic mice typically do poorly. On both tasks the transgenic mice were significantly worse than the nontransgenic mice ($P < 0.001$), with no benefits detectable from cimetidine treatment (Table 1).

Figure 1. Y-maze performance in transgenic and nontransgenic mice treated with cimetidine or vehicle. Mice were placed in a Y maze for 8 minutes and the sequence and number of arm entries were measured. The fraction of triads that included all three arms is presented as percent alternation in panel A. The total number of arm entries is presented in panel B. "VEH" (blue bars) are vehicle treated mice. "CIM" (red bars) are cimetidine treated mice. "Tg" indicates transgenic mice. There was a significant effect of genotype in panel A and a significant effect of treatment in panel B (see results). Values presented are mean ± s.e.m.

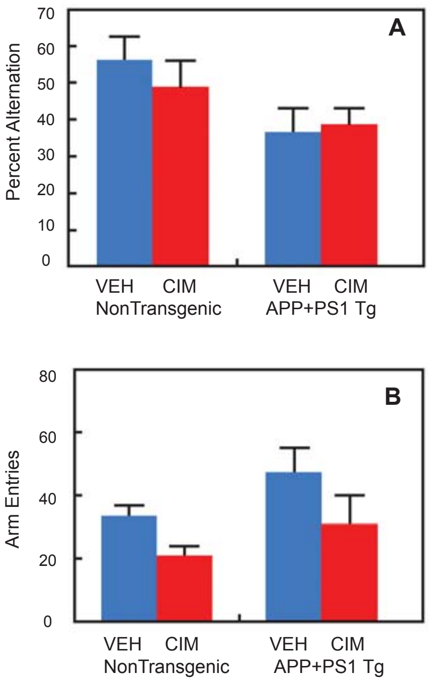
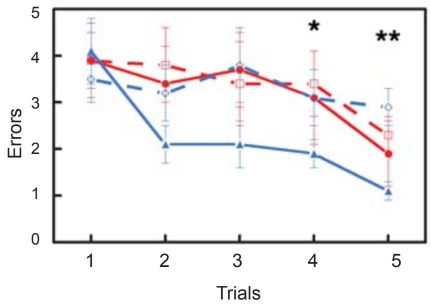


Figure 2. Radial arm water maze performance in transgenic and nontransgenic mice treated with cimetidine or vehicle. Mice were tested in a radial arm water maze. Data presented are the average of days 8 and 10 (day 9 day had to be discarded; see methods). Incorrect arm entries are presented as errors. Solid lines and symbols are for nontransgenic mice while dashed lines and open symbols are for transgenic mice. Vehicle treated mice are colored blue while cimetidine treated mice are colored red. Values are mean ± s.e.m. * $P < 0.05$ and ** $P < 0.02$ for transgenic versus nontransgenic vehicle treated mice (blue lines).



Histopathological measures

Mice were sacrificed and tissue processed for histopathology at 15 months of age. For measures of amyloid, only transgenic mice were analyzed. We found no effects of cimetidine treatment on Aß load in either hippocampus or in anterior cortex (Table 2). Similarly, we found no effects of cimetidine on Congo red staining, or on Thioflavine-S staining (table 2).

Cimetidine caused a significant activation of microglia in the

Table 1. Motor coordination in mice treated with cimetidine or vehicle.

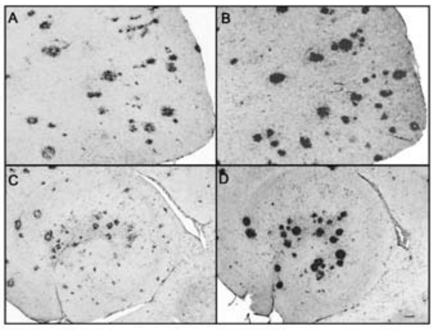
	Nontransgenic		Transgenic	
Task	Vehicle	Cimetidine	Vehicle	Cimetidine
Coat Hanger	40.9 ± 4.8	34.7 ± 6.9	16.8 ± 3.1	15.9 ± 3.6
Balance Beam	29.2 ± 9.8	24.8 ± 10.3	3.7 ± 0.5	3.0 ± 0.1

Value is time presented in seconds. Mean ± S.E.M.

Table 2. Amyloid and GFAP loads in transgenic mice treated with cimetidine or vehicle.

Measurement	Anterior Cortex		Hippocampus	
	Vehicle	**Cimetidine**	**Vehicle**	**Cimetidine**
Aß	21.6 ± 1.6	23.7 ± 1.9	22.7 ± 1.9	26.6 ± 1.3
Congo Red	0.74 ± .08	0.86 ± .08	0.81 ± .08	1.14 ± .15
Thio S	0.98 ± .12	0.86 ± .02	0.93 ± .09	0.82 ± .22
GFAP	19.1 ± 1.5	23.7 ± 1.9	8.9 ± 1.6	11.2 ± 2.0

Figure 3. CD45 immunohistochemistry of activated microglia in APP+PS1 transgenic mice following 12 months of cimetidine treatment. CD45 immunohistochemistry staining is shown in anterior cortex (panels A and B) and hippocampus (panels C and D). Panels A and C are from mice administered vehicle while panels B and D received cimetidine for 12 months. Magnification = 40X. Scale bar = 120mm.



APP+PS1 mice. A micrograph demonstrating this effect for the microglia marker CD45 is shown in Figure 3, where the microglial staining seen in vehicle treated mice (panels 3A, C) is lower than that found in mice treated with cimetidine (panels 3B, D), in both anterior cortex (panels 3A, B) and hippocampus (panels 3C, D). When the area occupied by reaction product was measured, there were roughly 80% increases in both anterior cortex ($P < 0.03$) and hippocampus ($P < 0.01$) of the mice administered cimetidine (Figure 4). This elevation was largely replicated with a second microglial marker for antigen CD11b (mac-1 antibody; Figure 5). The area occupied by reaction product staining, was increased 2 fold in the anterior cortex ($P < 0.001$). While a trend was present in the hippocampus for this marker to increase in cimetidine treated animals, it was not significant statistically ($P = 0.09$). This elevation in glial staining was restricted to microglia, as no changes in staining for the astrocyte marker GFAP were found in these same mice (Table 2). Furthermore, cimetidine treatment failed to modify any of the glial cell markers in nontransgenic mice treated for the same duration with cimetidine (values for CD45 are Cortex CTL=0.09±0.02, CIM=0.09±0.03; Hippocampus CTL=0.11±0.02, CIM=0.08±0.0; values for CD11b are Cortex CTL=9.22±2.3, CIM=10.2±2.9; Hippocampus CTL=14.2±1.7, CIM=16.5±2.1; values for GFAP Cortex CTL=0.05±0.01, CIM=0.04±0.01; Hippocampus CTL=1.6±0.13, CIM=1.7±0.23).

Discussion

The primary rationale for these studies, that H2-blockers might protect against AD by modifying amyloid metabolism in a manner that would protect from AD, is not supported by these results. Although it is possible that larger sample sizes might reveal a modest effect, the standard errors

Figure 4. Cimetidine results in increased CD45 staining in transgenic mice following 12 months of cimetidine treatment via drinking water. Data are expressed as the percentage of the measured area that is positively stained by immunohistochemical reaction product. "VEH" (blue bars) are vehicle treated mice. "CIM" (red bars) are cimetidine treated mice. Values are mean ± s.e.m. * $P < 0.05$; ** $P < 0.01$.

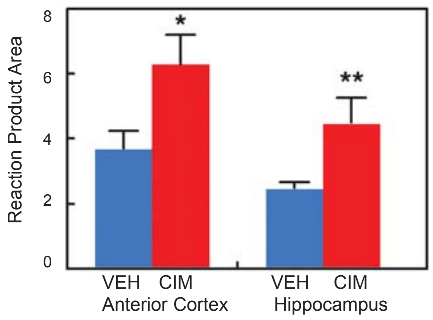


Figure 5. CD11b immunohistochemistry of microglia in APP+PS1 transgenic mice following 12 months of cimetidine treatment. CD11b (mac-1 antibody) immunohistochemistry staining is shown in anterior cortex (panels A and B) and hippocampus (panels C and D). Panels A and C are from mice administered vehicle while panels B and D received cimetidine for 12 months. Magnification = 40X. Scale bar = 120 mm.

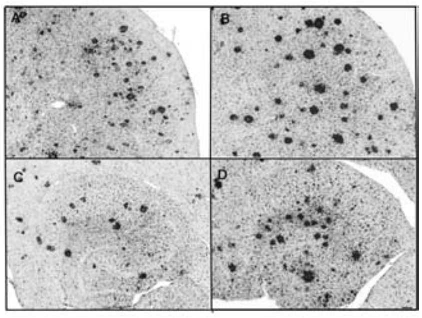
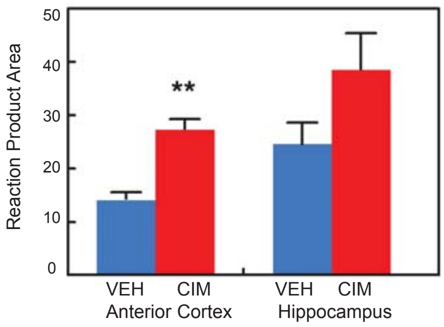


Figure 6. Cimetidine results in increased CD11b immunohistochemistry in anterior cortex following 12 months of cimetidine treatment via drinking water. Data are expressed as the percentage of the measured area that is positively stained by immunohistochemical reaction product. "VEH" (blue bars) are vehicle treated mice. "CIM" (red bars) are cimetidine treated mice. Values are mean ± s.e.m. ** $P < 0.01$.



for most of these measures were less than 10% of the means, suggesting 20-25% reductions could have been detected. If anything, the mean values suggest a slight increase in amyloid deposits in the cimetidine treated mice.

There are several possible explanations why cimetidine did not reduce amyloid loads in the transgenic mice. One explanation is that the potential benefit of H2-blockers in AD is at a step in pathogenesis other than amyloid deposition. Breitner et al.[5] argued that H2-receptor antagonists may decrease excitotoxicity. Histamine activation of H2-receptors can provoke hippocampal bursting activity[18], and activate the ERK2 signaling cascade[19]. Histamine can also potentiate the neuronal excitotoxicity mediated by NMDA receptor activation in vitro[20]. Given the apparent benefits of the low affinity NMDA receptor blocker memantine in mid and late stage AD patients[21], an anti-histamine action which diminishes NMDA receptor function may contribute to the therapeutic benefit of cimetidine. To our knowledge, no one has yet addressed the question of whether H2-blockade would diminish neural excitotoxicity in vivo.

Circulating histamine levels are elevated in AD, and increase further as the disease progresses; although controversial, this elevation may extend to CNS histamine as well[22]. Histamine is also toxic to endothelial cells in culture[23], suggesting this as another potential site at which antihistamine activity might protect individuals from AD.

A second possibility is that not all the drugs in the H2 blocker category confer protection against Alzheimer dementia. Some now argue that not all cyclo-oxygenase inhibiting NSAIDs will be effective in reducing AD risk because only some of the drugs in this class interfere with gamma secretase processing[24]. By analogy, H2-receptor blockade may not be the pharmacologic action of this class of agents which provides protection against AD. Possibly, one of the H2 blocking agents other than cimetidine has an amyloid modifying or other relevant activity that does not depend upon H2-blockade, and this agent contributes the bulk of protection in the epidemiologic studies. While we specifically chose cimetidine in part because it was less selective pharmacologically than other H2-antagonists, we cannot rule out this possibility.

A third option is that use of H2 antagonists are not protective against AD. While two studies found considerable support for use of these agents in lowering the risk or delaying the onset of dementia[25,26], other studies failed to find an association[27]. In a follow up to one of the earlier studies finding a benefit of H2 antagonists and NSAIDs on the prevalence of AD, NSAID use was further associated with reduced incidence of new cases, while no benefit regarding incidence was found with H2-blockers[28]. Finally, a 12 month trial found the H2-antagonist nizatidine was ineffective in slowing the loss of cognitive function in AD patients[29]. Resolution of this question will require controlled prevention trials with H2-receptor antagonists.

The major effect of cimetidine in this study was an enhanced activation of the microglia associated with the amyloid deposits in the transgenic mice. This increase approached two fold and was found in both hippocampus and frontal cortex, the two regions examined. This was not expected, as little work has been published evaluating the effects of histamine or H2-antagonists on brain-derived microglia. However, monocytes/macrophages derived from peripheral sources can be regulated by histamine and H2-antagonists in a manner consistent with the effects we observed in the brain.

For example, in peripheral blood mononuclear cells, histamine can diminish the lipopolysaccharide induced increases in tumor necrosis factor alpha production. This effect is blocked by H2-antagonists[30,31]. If a similar activity of cimetidine occurred in transgenic mouse brain, this may increase TNF-alpha production in microglial already induced by proximity to the amyloid plaque. Histamine is also reported to decrease synthesis of complement component C5 in cultured peritoneal macrophages in a cimetidine reversible manner[32]. Given the potential of amyloid to activate the complement cascade[3], cimetidine's abrogation of tonic histamine complement suppression may accelerate the inflammatory process in the mice. Finally, cimetidine can accelerate the transformation of peripheral monocytes into macrophages[33], a process analogous to the activation of resident monocytes, the microglia, in the CNS.

Thus, the literature on the peripheral regulation of monocytic cells by histamine is consistent with an activating effect of cimetidine. Recently, we and others have argued that some activation of microglia in the CNS may actually benefit disorders of amyloid deposition, at least in transgenic mice[34,35]. While certainly excessive microglial activation can be deleterious, to the extent that cimetidine promotes a beneficial state of microglial activation, it may also serve as a mechanism by which this drug reduces the risk of developing AD.

Acknowledgements

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ARTICLE

Electron Transport Chain Complex II-III Activity is Decreased in Alzheimer's Disease Skeletal Muscle

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Abstract

An increasing number of studies have indicated that a disturbed mitochondrial function is involved in Alzheimer's disease (AD) pathogenesis. Mitochondria contain the electron transport chain comprising four different enzyme complexes: Complex I, II, III and IV. In the present study biochemical studies of complex I+III, II+III and IV activities, correlated to the activity of the mitochondrial marker enzyme citrate synthase (CS), were performed together with studies of mitochondrial morphological and histochemical properties in skeletal muscle tissue from 10 AD patients and 9 control persons. Neurogenic changes were present in biopsies from both AD patients and controls as a result of an age-related denervation-reinnervation process, which seemed slightly more pronounced in the AD group. No convincing intergroup differences could be discerned in mitochondrial enzyme-histochemical stainings. In contrast, we found that complex II+III activity in the biochemical studies were decreased in AD skeletal muscle as compared to controls (0.49±0.03 units/unit CS [mean±SEM] vs. 0.59±0.03 units/unit CS, p<0.05). There were no significant differences in complex I+III or IV activity. These results indicate a mitochondrial disturbance in AD skeletal muscle and emphasize further that AD is not confined to the brain.

Keywords: *Electron transport chain, mitochondria, skeletal muscle, Alzheimer's disease, succinate-cytochrome c reductase.*

Introduction

Mitochondrial degeneration is an important part in Alzheimer's disease (AD) neuropathology[6,19,22,24,35,50,59] that appears early in the disease before tangle formation[21]. Mitochondrial dysfunction can result in increased free radical production, impaired intracellular calcium homeostasis and increased vulnerability to excitotoxins[31,39], all mechanisms which have been implicated in AD pathogenesis[32,46,52].

Mitochondria are the main sources of energy in cells and contain the electron transport chain involved in oxidative phosphorylation. The electron transport chain is located in the inner mitochondrial membrane and comprises four different enzyme complexes: Complex I (NADH: ubiquinone oxidoreductase), complex II (succinate: ubiquinone oxidoreductase), complex III (ubiquinol: cytochrome c reductase) and complex IV (cytochrome c oxidase, COX). These complexes, together with a fifth enzyme complex (complex V

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[ATP-synthase]), are all involved in oxidative phosphorylation in the cell, i.e. the transfer of electrons to oxygen coupled to ATP synthesis.

Disturbances in the mitochondrial electron transport chain in the AD brain have been reported with changes in complex IV being the most consistently found. Thus, decreased COX activity and mRNA levels of mitochondrial-encoded COX subunits have been shown as well as changed COX kinetic behaviour and levels[8-9,12,25,33,38,51]. In peripheral tissues a decreased COX activity has been found in cultured fibroblasts from sporadic AD patients[16] and platelets[9,42-43]. In cell lines depleted of endogenous mitochondrial DNA and repopulated with platelet mitochondrial DNA from Alzheimer disease patients and thus creating a cellular model of the disease in so-called cybrids[28], COX activity was significantly depressed[54]. Dysfunctions in complex II+III and I have been reported also with a decreased activity being found in mitochondrial preparations from AD temporal cortex[20] and occipital cortex[38], as well as a reduction in the FeS subunit of complex III in AD hippocampus[8]. A decreased gene expression of mitochondrial DNA-encoded ND4, a subunit of complex I, have been reported in AD temporal cortex[18].

Furthermore, mitochondrial function may be impaired by amyloid β (Aβ) peptide[1,11,44-45,49]. Aβ is a proteolytic fragment of the larger amyloid-β precursor protein (βAPP) and pathologically accumulated in the cerebral cortex and blood vessel walls in the AD brain. Aβ has been identified also within skeletal muscle fibers in inclusion-body myositis (IBM)[3] and recently in Alzheimer disease temporal muscle with elevated levels of amyloidogenic Aβ1-40 and Aβ1-42 peptides being found as compared to control muscle tissue[27]. Although giving different clinical symptoms, AD and IBM have been suggested to share the same pathogenic mechanisms[4], both being slowly progressive age-related disorders and with a remarkable similarity in their molecular phenotype including accumulation of Aβ and phosphorylated tau protein, inflammation and signs of mitochondrial disturbances and oxidative stress, albeit in different organs[3]. Brain and skeletal muscle are both tissues highly dependant on normal oxidative metabolism and mitochondrial function.

With this in mind and to look for a possible marker for AD, we have studied mitochondrial function in AD skeletal muscle tissue by studying the activity of mitochondrial complex I+III, II+III and IV together with mitochondrial morphological and histological properties in skeletal muscle tissue from AD patients.

Materials and methods

Materials

All chemicals were obtained from the Sigma Chemical Co., St Louis, Mo, USA.

Subject and tissue details

After approval by the local Ethical Committe and informed consent from control subjects and patients or their relatives, 50-100 mg of muscle tissue were taken percutaneously in the tibial anterior muscle with percutan biopsy technique ad modum Radner[47] from 10 AD patients (8 females and 2 males, mean age± SEM 81±1.6, range 70-86 years) and 9 control persons (2 females and 7 males, mean age ± SEM 75±1.7, range 68-82 years). All patients had a clinical diagnosis of probable AD according to NINCDS/ADRDA[34].

Control persons with no dementia disease in their family history were evaluated via an extensive investigation including MRI, SPECT, EEG and neuropsychological and laboratory tests with no history or clinical signs or symptoms of neurological or psychiatric disease being found.

Subjects with ongoing infections, excessive alcohol consumption, diabetes, kidney or muscle disease or any other serious illness were excluded. All subjects were in good physical health and the degree of physical activity in the individuals was appraised to be equal between the two groups.

Histochemical and morphological analyses

Muscle biopsy procedure

The biopsy material used for light microscopy including morphological and histochemical analyses was quickly frozen in freon-22, cooled with liquid nitrogen (-190°C), and subsequently stored in a freezer at -75°C until further processed. Sections of 10-15 µm were cut in a cryostat operating at -20°C and stained with haematoxylin-eosin, modified Gomori trichrome[17], and for myosin-adenosin-triphosphatase (myosin-ATPase)[10,40], NADH-TR[48], COX, succinate dehydrogenase (SDH), and for lipid (Oil red O, Sudan Black) and glycogen content (PAS).

The muscle fibre nomenclature was based on the stainability for myosin-ATPase. Thus, fibres with high content of acid-stable myosin-ATPase and low content of alkali-stable myosin-ATPase were termed "type I" while fibers with the reversed staining patterns were termed "type II"[10]. All fibers on a muscle biopsy cross-section were classified and the total number of each type estimated.

Blinded morphological evaluation was made by two myologists (TA and KB), according to routine procedures.

Biochemical analyses

Mitochondria were isolated from fresh muscle tissue as previously described[58]. The activities of the mitochondrial enzymes were determined spectrophotometrically at 25°C. An aliquot of the isolated mitochondria was freeze-thawed in hypotonic media[5], and the activities of rotenone sensitive NADH-cytochrome c reductase (NCR) and succinate-cytochrome c reductase (SCR) were determined according to Sottocasa et al.[53] and Cooperstein et al.[14], respectively. For the determination of COX activity the isolated mitochondria were freeze-thawed once, and treated with digitonin 2 mg/mL before the determination of COX[15].

The activity of citrate synthase (CS) was determined a) in whole muscle after homogenization in a medium containing Triton X 100 0.05%, K_2HPO_4 50 mmol/L and b) in isolated mitochondria permeabilized in the same medium[2].

All enzyme activities (except CS activity in whole muscle) were measured as mmol/min/L mitochondrial suspension.

Data were analyzed by Mann-Whitney's U test. Regression analysis was used to investigate possible correlations between age and enzyme activities.

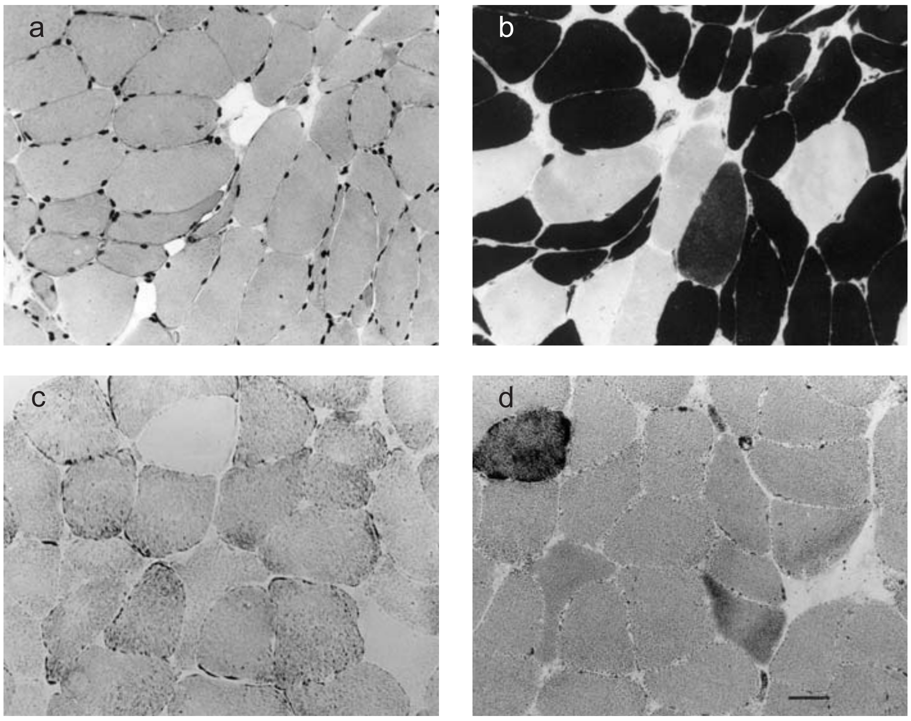

Figure 1. Muscle biopsy from m. tibialis anterior of a patient with Alzheimer's disease. Cross-sections were stained with Htx-eosin (a), for myofibrillar ATPase after acid preincubation at pH 4.6 (b), and for COX (c) and SDH (d). In (a) and (b), there are small groups of angulated atrophic fibres of the same histochemical fibre type (type I). In (c), two fibres with lack of staining for COX are seen. These fibres correspond to fibres with increased staining for SDH (d). Bar=0.05 µm.

Results

Morphological and enzyme-histochemical analyses were performed on 9 of the AD patients and 8 of the control subjects (Table 1). In the Alzheimer group, muscle biopsies exhibited a slight variation of fibre size in 7, and a moderate variation in 2 patients. Three of the muscle biopsies contained central nuclei. Atrophic muscle fibres, either scattered or in groups, were seen in 8 patients (Fig. 1), and type grouping (defined as the occurrence of groups of both type I and II fibres, in which fibres of one histochemical type are surrounded only by fibres of the same type) in 5.

In all patients there was a predominance of type 1 muscle fibres. NADH-TR staining was normal in all but 2 biopsies in which some muscle fibres had a moth-eaten appearance, as a sign of structural changes within the fibres. Two of the biopsies contained sporadic ragged red fibres, as seen in the Gomori trichrome and SDH stains. COX staining was normal in all but one patient in which a loss of staining was seen in a few muscle fibres, which corresponded to increased SDH staining (Fig. 1). Increased SDH staining was also found among a few, scattered muscle fibres throughout the biopsies in all but one of the patients. There were no alterations in stainings for fat or glycogen content in any of the muscle biopsies.

In the controls, a slight variation of muscle fibre sizes were seen in 5, central nuclei in 2 and atrophic fibres, either scattered or in groups, in 3 biopsies, respectively. Type grouping as defined above, was presented in 2 muscle samples. There was a type 1 fibre predominance in all control biopsies. Stainings for NADH-TR, and for fat and glycogen were normal in all controls. A few COX negative and ragged red fibres were seen in 1 control. SDH staining was increased in scattered muscle fibres in four of the controls.

Figure 2 shows the respiratory chain enzyme activities per unit CS activity in a suspension of isolated mitochondria. A significant decrease in complex II+III activity was found in AD patients (n=10) with a mean reduction of 21.1% as compared to controls (n=9). SCR activities were 0.49±0.03 (mean±SEM) and 0.59±0.03 units/unit CS, respectively, $p<0.05$ (Fig 2). With a cut-off of 0.58 or less, the sensitivity is 90% (9/10), the specificity 77.8% (7/9) and the positive predictive value 81.8%.

There were no significant differences between the two groups in the enzyme activities of complex I+III (NCR: 0.56±0.06 and 0.57±0.03 units/unit CS, AD patients and controls, respectively) and IV (COX: 1.42±0.10 and 1.65±0.07 units/unit CS, respectively) or in enzyme activities when correlated to each other (Fig. 2).

The activity of the mitochondrial matrix enzyme citrate synthase was determined also in total muscle tissue with no significant differences

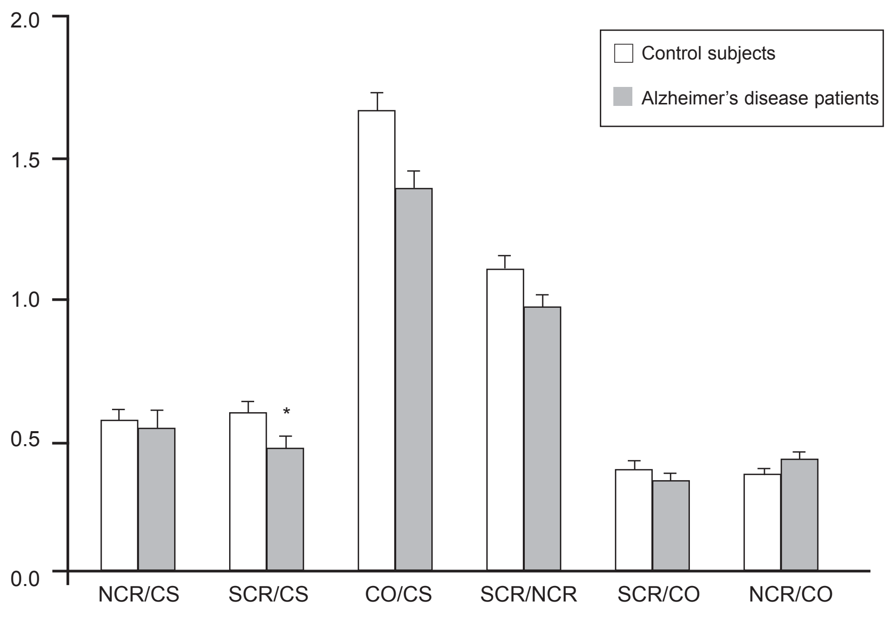

Figure 2. Enzyme activities in isolated mitochondria from muscle tissue. All enzyme activities were measured as mmol/min/L mitochondrial suspension. Data are means ± SEM, n=10 (Alzheimer's disease patients) or 9 (control subjects). *p<0.05, Mann-Whitney's U-test.

being found between AD patients and controls (17.7±1.5 mmol/min/kg muscle tissue and 16.8±1.1 mmol/min/kg muscle tissue, respectively).

Although AD patients and control persons were of similar ages, statistical analyses showed a significant difference in age between the two groups ($p<0.05$). However, this was not considered to be a complicating factor in the study, since regression analyses of activity per unit citrate synthase for the enzymes NCR, SCR and COX versus age for either AD patients or controls, separately or together, showed no significant effect of age in the present samples ($p>0.13$). The same was true for citrate synthase activity in total muscle tissue or mitochondrial suspension ($p>0.11$).

Discussion

In the present study, the activities of the enzyme complexes I-IV in the mitochondrial electron transport chain have been investigated in isolated mitochondria, together with mitochondrial morphology and histology in muscle tissue from AD patients and controls. It was found that SCR (complex II+III) activity is significantly reduced in AD muscle tissue.

Muscle fibre abnormalities of neuropathic type were seen in 6 out of 9 Alzheimer patients and in 4 of 8 controls. Age-related degenerative neuronal changes and decrease in the number of myelinated nerve fibres have been observed in various peripheral nerves and spinal roots of many species, including man[29]. Neurogenic changes as seen in the present study is therefore to be expected as part of an age-related denervation-reinnervation process. Whether the degree of neurogenic changes in the peripheral nervous system of Alzheimer patients differs from that of age-matched controls has not been studied in detail. In the present study, the neurogenic changes seemed more frequent and more pronounced in the Alzheimer group, but definite conclusions cannot be made from these qualitative evaluations due to the limited number of patients and controls.

An association between ageing and an increase in the number of COX-deficient muscle fibres has been reported,[37] as well as a decline in both respiration rate and enzyme activities of complex I and IV in human skeletal muscle[53]. The present enzyme-histochemical analyses revealed sporadic fibres with an increased SDH staining of muscle fibres in both Alzheimer patients (8 out of 9) and controls (4 out of 8). This may at first glance fit with our biochemical findings of a disturbed enzyme activity of complex II+III in the Alzheimer group. Again, however, the enzyme-histochemical observations must be interpreted with caution due to the limited number of subjects and since generally only a very few fibres in each group exhibited abnormal stainability. Thus, no intergroup difference could be established with certainty regarding the SDH staining.

Fibres with an abnormal (negative) staining for COX were found only in sporadic fibres and in only one individual of each group. This finding is in accordance with our biochemical data in which no statistically significant group difference was found for enzyme activities of complex IV.

The biochemical measurements of the respiratory chain enzymes were performed in a suspension of mitochondria purified from fresh muscle tissue, and were related to the activity of CS, determined also in the mitochondrial suspension. Since CS is specifically located to the mitochondrial matrix, we choose it as a reference to the measurements, rather than protein content in the mitochondrial suspension which may contain protein residues of non-mitochondrial origin. The activity of CS was determined also in fresh muscle tissue, with no changes being found between AD patients and controls in agreement with other studies on brain[9,33,38], platelets[9] and lymphocytes[36]. This indicates that the decreased SCR activity per unit CS in the AD cases found in this study is not due merely to a loss of mitochondria but reflects a specific change in SCR activity in AD skeletal muscle.

Table 1. Morphological alterations in muscle biopsies from patients with Alzheimer's disease (n=9) and controls (n=8).

ALZHEIMER PATIENTS	Gender	Age	Variation in fibre size	Atrophy	Hypertrophy	Fibre typ distribution	Type grouping	Centralized nuclei	Other	OVERALL EVALUATION
2443 (AS-11)	Female	86	+	+	0	I > II	no	0	0	Normal
2442 (MH-17)	Female	80	++	+/++	0	I > II	yes	0	Increased SDH activity in 7 fibres; 2 COX-negative fibres	Slight neuropathy (+) Signs of mt dysfunction
2444 (HB-20)	Female	77	+	+	0	I > II	yes	0	Increased SDH activity in 7 fibres	Slight neuropathy (+) Signs of mt dysfunction
2462 (GA-27)	Female	70	+	+	0	I > II	0	0	Increased SDH activity in 6 fibres; 1 ragged-red fibre in GT; Moth-eaten fibres	Signs of mt dysfunction
2463 (FS-14)	Female	83	+	+	0	I > II	0	+	Increased SDH activity in 9 fibres	Signs of mt dysfunction
2464 (SH-12)	Female	85	++	++	0	I > II	yes	0	Increased SDH activity in 10 fibres;	Moderate neuropathy (++) Signs of mt dysfunction
2559 (AL-13)	Female	85	+	++	0	I > II	yes	0	Increased SDH activity in 1 fibre	Moderate neuropathy (++)
2628 (NS-15)	Male	83	+	+	+	I > II	0	+	Increased SDH activity in 6 fibres; 1 ragged-red fibre in GT; Moth-eaten fibres	Moderate neuropathy (++) Signs of mt dysfunction
2629 (KE-22)	Male	76	+	0	0	I > II	yes	+	Increased SDH activity in 1 fibre	Slight neuropathy (+)
CONTROLS										
2473 (AB-15)	Male	82	+	0	0	I > II	0	0	0	Normal
2474 (SR-29)	Male	68	+	+	0	I ≈ II	0	0	Increased SDH activity in 5 fibres; 2 COX-negative fibres; 1 ragged-red fibre in GT	Slight neuropathy (+) Signs of mt dysfunction
2488 (ÅÅ-18)	Male	79	+	+	0	I > II	yes	+	Increased SDH activity in 7 fibres	Slight neuropathy (+) Signs of mt dysfunction
2477 (LG-18)	Male	79	+	0	0	I > II	0	+	Increased SDH activity in 1 fibre	Normal
2476 (DO-27)	Male	70	Normal	0	0	I > II	0	0	0	Normal
2479 (AJ-25)	Female	72	Normal	0	0	I > II	0	0	0	Normal
2480 (KH-25)	Female	72	Normal	0	0	I > II	0	0	0	Slight neuropathy (+)
2478 (GG-25)	Male	72	+	+	+	I > II	0	0	Increased SDH activity in 4 fibres	Slight neuropathy (+) Signs of mt dysfunction

SDH = succinate dehydrogenase; COX = cytochrome c oxidase, GT = modified Gomori trichrome + = slight, ++ = moderate

A significant decrease in SCR activity with age in human muscle tissue has been reported by Trounce et al.[56], whereas we found no correlation. Our results are in agreement with other studies[7,13], including Brierley et al. who found little correlation between age and mitochondrial respiratory activity in subjects matched for physical activity[7]. Different subject selection and methods may account for this, since Trounce et al. obtained muscle tissue from patients undergoing surgery or suffering from chronic fatigue or muscle pain syndrome, and assayed enzyme activities not correlated to CS activity in homogenized muscle tissue. In the present study, all subjects were estimated to exercise the same amount of daily physical activity including daily walks. In the study by Brierley et al., complex I and IV but not complex II activity was affected by physical activity and no differences in electron transport chain activities between the sexes were found[7]. This indicates that the decreased complex II+III activity in the present study is not merely due to different degrees of physical activity or sex differences.

The decreased SCR activity reported in the present study concur with findings in AD brain in temporal[20,38] and occipital cortex[38], where a 21-30% decrease of complex II+III activity was found. In contrast, in a previous study on AD muscle tissue, Mariani et al. showed significantly increased activities of several oxidative enzymes including SCR [+80%] and COX [+37%]) in AD muscle tissue[30]. However, this was not convincingly confirmed in their histological analyses where an increased COX and SDH activity was found in three out of ten AD patients[30]. Again, methodological differences but also different subgroups of AD patients might explain discrepant results on electron transport chain dysfunctions in AD, as has been reviewed by Kish et al regarding COX activity in AD brain[26].

Since we did not find any changes in NCR (complex I+III) activity, together with our histochemical findings of a possibly changed SDH (complex II) activity within the AD patients group, it is tempting to suggest that the decreased SCR activity in AD skeletal muscle is due to a complex II disturbance and/or deficient electron transport between complex II and III. The possible consequences of a complex II disturbance in AD has been demonstrated by Keller et al who reported an increase in intracellular calcium levels, apoptosis and accumulation of reactive oxygene species after complex II inhibition in PC12 cells expressing mutant presenilin-1[23].

Whereas we could find no evidence of a disturbed COX function neither in our morphological nor in our biochemical experiments, several studies have reported a deficient COX activity in AD non-neural tissues as well as in AD brain. However, data are still conflicting since not all groups have been able to find a disturbance, and there is still confusion regarding which brain regions are involved. Our data are in agreement with those of Gu et al. who found a decrease in complex II+III but not complex IV activity in AD temporal cortex, an area usually severely affected in AD[20].

The presence in this and other studies of changed respiratory chain complex activities outside of brain suggests the possibility of a genetic defect, inherited or acquired, as an underlying cause of the mitochondrial disturbances found in AD. However, hitherto it has not been proved or disproved whether a mitochondrial DNA defect contributes to AD pathogenesis (for review see ref 55). An alternative explanation could be the influence of Aβ peptides. Aβ25-35 and Aβ1-40 have been shown to affect mitochondrial function, with a decreased complex II+III and complex IV activity in PC12 cell cultures[44-45] and complex IV activity in rat brain mitochondria[11] being reported. Since AD muscle contains significantly elevated levels of Aβ1-40 and Aβ1-42[27], one possible explanation for our findings is that they are a consequence of Aβ effect on the electron transport chain, in analogy with inclusion body myositis where mitochondrial abnormalities including COX negative muscle fibers has been suggested to be induced by Aβ and/or βAPP[4]. However, in contrast to IBM patients, AD patients do not suffer from muscle weakness and atrophy but stay relatively unaffected in their musculo-skeletal status until the later stages of the disease. One reason for this may be that the AD skeletal muscle changes in Aβ levels as well as in complex II+III activity in the present study are of a relatively small degree. As the disease progresses with time these changes might accumulate and together with an augmented generation of reactive oxygen species finally give clinical symptoms.

In conclusion, mitochondrial morphology and mass as well as complex I+III, II+III and IV enzyme activities in the mitochondrial electron transport chain have been investigated in AD skeletal muscle. It was found that SCR (complex II+III) enzyme activity was decreased in AD. The decrease was, however, too small to be suitable as a biological marker for AD. Our findings support the evidence of there being a mitochondrial disturbance in AD, not confined to the brain.

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Abbreviations

Alzheimer's Disease	AD
Amyloid β	Aβ
Amyloid-β precursor protein	βAPP
Citrate synthase	CS

Cytochrome c oxidase	COX
Inclusion-body myositis	IBM
NADH-cytochrome c reductase	NCR
Succinate-cytochrome c reductase	SCR
Succinate dehydrogenase	SDH

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