Editorial

Hypothesis-driven research and the omic era. Weinstein's synergism.

The amassing of enormous data sets in genomics, proteomics and imaging has led a number of scientists to envision a future in which informatics concepts and tools will eventually rival the traditional hypothesis-driven research that has dominated biomedical science for at least the past century. Despite the skepticism of prominent scientists such as J.F. Allen (2001) among other, in the opposite camp of the debate Neil R. Smalheiser believe that informatics tools can assist investigators to formulate, assess and prioritize their hypotheses. Actually, the bio-medical research entered the omic era. The recently growing field of omics and the omics approach is not at all a sophisticated "translation" of the usual language in esoteric terms, but a newly proposed methodological tool that strives to put a new conceptual order in the scientific thinking by performing an integrative attempt, able to reveal the intimate core of a given scientific approach and to drive it to practice improvement. As the scientists are increasingly integrating bio-medicine with information science, they took up the use of omics, widely applied by the bioinformaticians and molecular biologists. In the USA there are perhaps the largest number of bioinformatcians and bio-medical researchers who successfully use various omics such as phenome, physiome, metabolome, and so on. Since large-scale omic studies of cellular molecules in aggregate rarely can answer critical questions without the assistance of information from traditional hypothesis-driven research, the two types of scientific approach must be synergic, as point out John N. Weinstein in 2001. For the bio-medicine researchers omics concepts provide a new, holistic paradigm of doing biological research, possible heuristic skeletons for various less well defined fields, so becoming one of the most convenient and extensive reformations of biology. At Ana Aslan International Academy of Anti-Aging our scientific research team just elaborated and putted under work two such research paradigms/projects, already welcomed and included in an international scientific cooperation framework. One of them is centered on the omic (genomic/epigenomic, metabolomic/methylomic, nutriomic and sociomic) approach of Mild Cognitive Impairment – MCI, a pathological entity of maximum interest because of its quality of a stage still susceptible to successful medical and non-medical interventions, and to preventive algorithms. The second one deals with the reconsideration of certain old pharmaceutical and non-pharmaceutical members of the brain ageing-delayers and brain pathology correctors' therapeutic arsenal. The definition and the study of the critical omes in brain aging and neurodegenerative pathology could be useful, holistic methodological attempts, able to answer critical questions or to fruitfully reconsider old theories, research outcomes and medical interventions.

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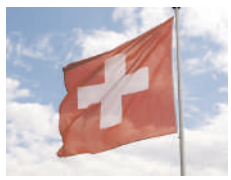

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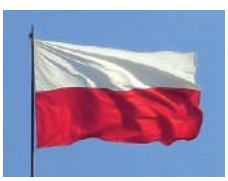

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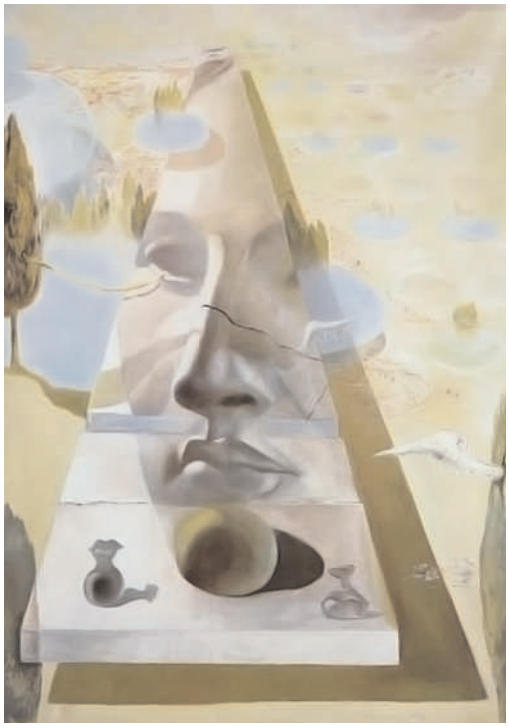

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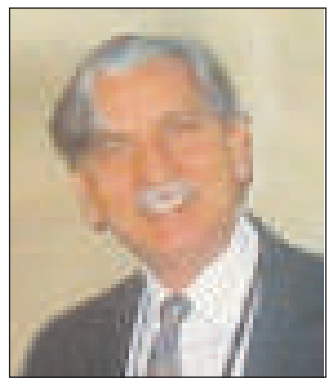

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Rivastigmine In Alzheimers Disease: Cognitive Function And Quality Of Life

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Abstract

Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterised by a progressive loss of cognitive and functional abilities, associated with a various degree of behavioural disturbances, with a devastating impact on public health and on the whole society.Slowing of cognitive impairment, duration of disease, self-sufficiency, behavioural disturbances represent the best outcomes of the pharmacologic therapy.Cholinesterase inhibitors (ChE-I) have been shown to be effective in the treatment of the cognitive, behavioural, and functional deficits of AD. Rivastigmine is a dual inhibitor of both acetylcholine esterase (AChE) and butyrylcholinesterase (BuChE), enzymes involved in hydrolysis of Ach. This drug has been shown to benefit patients with AD, however, the benefits are limited and their long-term effectiveness has not been well-demonstrated.

Keywords: Alzheimer disease, Anti-cholinesterase, Cognitive impairment, Rivastigmin, Alzheimer, Drugs, Therapy.

Introduction

Dementia of Alzheimer's type (AD) (Mc Khann et al. 1984) is a chronic neurodegenerative disorder characterized by an insidious onset and a progressive loss of cognitive and functional abilities, associated with a various degree of behavioral disturbances, progressively leading to a total dependency. Alzheimer's disease is the most common form of dementia, accounting for 50–60% of all cases. The prevalence of dementia is below 1% in individuals aged 60–64 years, but shows an almost exponential increase with age, so that in people aged 85 years or older the prevalence is between 24% and 33% in the Western world (Ferri et al 2005) Representative data from developing countries are sparse, but about 60% of patients with dementia are estimated to live in this part of the world. Alzheimer's disease is very common and thus is a major public health problem. In 2001, more than 24 million people had dementia, a number that is expected to double every 20 years up to 81 million in 2040 because of the probable increase in life expectancy (Ferri et al 2005). Since the AD has become a major health and economic burden to society several efforts are direct to develop a therapeutic strategy in order to modify the natural history of AD. Generally, AD has a mean duration of 6 - 10 years: the cognitive annual loss measured with the Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS Cog) (Rosen et al 1984) is 8 - 10 points, with the Mini Mental Status Examination (MMSE) (Folstein et al 1975) is 2 - 4 points; the Clinical Rating and Clinician's Interview-Based Impression of Change Plus Caregiver Input (CIBIC-Plus) (Scneider et al 1997) reports a six-month decline of about 1,5%. Note that these scales are the most utilised in the assessment of cognitive disorders. Approximately, 4- 6 years elapses between the total autonomy and the total functional dependency of AD patients. The behavioural disturbances are present at least in 90% of the patients and, depending from environmental variables, with a variable incidence in the various stages, in different individuals and in the same patient at different times. The duration of disease, leading to total dependency, means an important number of years for patients, relatives and care givers with an unsatisfactory and poor quality of life; the latter represents therefore one of the most important outcomes of the pharmacologic therapy.
Currently two classes of drugs, cholinesterase inhibitors and NMDA receptor antagonist, are recommended for the symptomatic treatment of AD, each targeting a different neurochemical component thought to underlie the condition. The cholinesterase inhibitor are widely recommended for the treatment of mild to moderate AD (Doody et al 2001; Davies et al 1976; Ballard 2002). In 2004 the first NMDA receptor antagonist (memantine) was approved for the treatment of moderate to severe AD. These drugs may also improve the ability to remain independent, reduce the likelihood of admission to residential/nursing care and improve carer health-related Quality of Life (QoL).
These therapeutic indications and the guidelines for treat-

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ment (Davies et al 1976; Ballard 2002) mainly derive from randomized controlled trials (RCT). Despite the methodological doubts about the large clinical use of data derived from an experimental context, these indications are based on the only proven evidences of an effective use of pharmacologic therapy in dementia. This review aims to present an overview of the clinical effectiveness of the symptomatic treatments of rivastigmine for people suffering from (mild to moderately severe) Alzheimer's disease.

Assessment

To determine prognosis for an illness such as AD, in which there is a continuous neurodegenerative process, it is useful to assess how rapidly the disease is progressing clinically and how severely the patient is currently affected. Historically, AD severity was defined most often by the degree of cognitive impairment, of global function and by the presence of behavioural disturbances. In order to obtain such information and to assess the progress of the disease are used a standardized rating scales. In this regard, instruments such as ADAS-Cog and MMSE were used to provide a measure of cognitive impairment. However, it has been recognized that the degree of functional impairment also reflects AD severity. The Global Deterioration Scale (GDS) (Reisberg et al 1997) was designed specifically to evaluate AD severity by measuring cognitive and functional performance. In addition, scores on an instrument such as the Progressive Deterioration Scale (PDS) (Dejong et al 1989) also provide an index of disease severity; they reflect the ability of the patient with AD to perform specific instrumental and basic activities of daily living, which become increasingly compromised as the disease progresses.

Cognitive Function

ADAS-Cog represents a primary instrument specifically designed to assess cognitive function in AD, and has been shown to be valid and reliable. It assesses various cognitive abilities including attention, memory, orientation and language ability. The score range is 0-70 with higher scores indicating poorer functioning. MMSE is in another measure used to evaluate cognitive performance, this scale assesses multiple cognitive abilities: orientation, immediate recall, attention and calculation, delayed recall, and language. The score ranges is from 0 (severe impairment) to 30 (normal).

Global Assessment

CIBIC-Plus provides a global rating of patient function in four areas: general, cognitive, behaviour and activities of daily living. The CIBIC-Plus was based on interviews of both the patients and the patient's caregiver. The score range is on a scale of 1-7, with 1 showing marked improvement, 7 marked worsening with 4 representing no change. GDS is a global rating of overall dementia severity. It was developed for the assessment of primary degenerative dementia and the delineation of the stages of disease. The stages are scored from 1 (no cognitive decline) to 7 (severe cognitive decline).

Activities of daily living

PDS is a disease specific measure of changes in 29 items of the activities of daily living. It is a 100 point bipolar visual analogue scale that, based on caregiver input, measures the ability of patients to perform various activities of differing complexity; a higher score represents better functional ability. The interview is conducted with the caregiver. The Nurses' Observation Scale for Geriatric Patients (NOSGER) (Spiegel et al 1991) is used to assess various cognitive functions and behaviour as related to activities of daily living (self-care, disturbing behaviour, instrumental activities of daily living, memory, mood, social behaviour). The NOSGER questionnaire is completed by the next of kin or carer having most frequent contact with the patient.

Alzheimer Disease and Cholinergic Hypothesis

Since Davies and Maloney (Bartus et al 1982) first proposed the "cholinergic hypothesis", a large body of evidence has grown to support the view that impairment of cholinergic function is of central importance in the pathogenesis of AD (Gallagher et al 1995; Kasa et al 1997; Whitehouse et al 1982; Katzman 1986; O'Brian et al 2001). In patients with AD, cholinergic neuronal loss is particularly noticeable in the neocortex and hippocampus. These areas of the brain are associated with learning and memory, executive functioning, behaviour and emotional responses (Cummings 2000). Building upon these studies, a number of therapeutic approaches were developed with the aim of enhancing cholinergic function, the most successful of which has been the use of cholinesterase inhibitors. Various experimental data evidence an interaction between the cholinergic deficits and the formation of amyloid plaques and neurofibrillary tangles: the in vitro modulation of the cholinergic system has a neuroprotective effect. There are some preliminary evidences in support of a neuroprotective effect for cholinesterase inhibitors, derived from studies on human cells, and rat brains and cells. In human beings affected from AD, the muscarinic agonists modify the liquoral concentration of -amyloid (Borroni et al 2001) In vitro studies demonstrate that ChE-I have the capacity to stimulate -amyloid aggregation and fibril formation: a further possibility to in-

terfere with the -amyloid formation may be the inhibition of the so called G1 form of ChE, the occurrence of which seems to be positively related with the density of -amyloid. More recently, administration of ChE-I as been shown to increase the ratio of Amyloid precursor protein (APP) forms in platelets of patients with AD. APP forms with apparent molecular weights of 130, 110, and 106 kd are present in human platelets. It has been demonstrated that AD is specifically associated with a decreased APP forms ratio in platelets. This data represent the first published findings in human being, suggesting a potential effect of ChEI on APP trafficking or processing in a peripheral cell: treatment with donepezil increases the ratio of APP isoforms (Polinsky 1998; Corey-Bloom et al 1998).

Evidence from animal studies suggests that rivastigmine is a more potent inhibitor of acetylcholinesterase in the cortex and hippocampus, the brain regions most affected by AD (Tariot et al 2000). Large multicentre trials have been completed in USA, Canada, Europe, Australia and South Africa.

Cholinesterase inhibitors (ChE-I) had been used initially in order to improve memory and cognition; later on, they had been tested with regard to other objectives of AD treatment: to improve functional level and patient's and caregiver's quality of life; in other words, to modify behavioural and cognitive status in a clinical meaningful way (Doody et al 2001; Davies et al 1976; Ballard 2002).

Rivastigmine

Rivastigmine tartrate is a carbamate pseudo irreversible inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), and selectively inhibits in the central nervous system as demonstrated using cerebrospinal fluid ChE activity The pharmacokinetic and pharmacodynamic characteristics of rivastigmine are presented in Table 1.

Rivastigmine is supplied in capsules and solutions for oral adminitration adsorbed rapidly after oral adimistration in healthy adults: in AD patients no difference was found with age. Taking rivastigmine with food slows the absorption and increase tolerability, because the gastrontestinal adverse effetcs are associated with high plasma levels. Rivastigmine binds to both the esterasic and ionic sites of AChE, preventing the enzyme from metabolizing Ach, but dissociated much more slowly than AchE ("pseudoirreversible" action"). It is metabolized by AChE and BuChE at the synapse: elimination is approximately complete 24 h after administration, with most eliminated renally (Williams et al 2003). Relevant pharmacokinetics drug-drug interactions are unlikely because rivastigmine has low protein binding and is not metabolized via hepatic CYP system as other two IchE donepezil and galantamine. No clinically significant interactions with 22 classes of concomitant medications was found in pooled data from RCT (Grossberg et al 2000). Adverse effect in trials are mainly cholinomimetic gastrointestinal symptoms predominant in the titration phase, included nausea (17-48%), vomiting (16-27%), and diarrhea (11-17%), minimized with slowly dose increase (every month) in clinical practice and assumption with food. The cholinomimetic theoretical risk of bradicardia especially in elderly patients was no dimonstrated in clinical trial as any signficant cardiac function effect. Central nervous system side effecs as headache (19%) and dizziness (20%). Adverse effects are more common among subjects taking higher dose with forced titration and primarily affected the G1 system: in general the subjects manifesting any side effects are about 13% with much lower incidence in the maintenance phase. Peripheral G4 side effects as muscolar fasciculations or cramps are rare (Desai et al 2005). A large meta-analysis of 16 randomised controlled trials documented the tolerability of AChE inhibitors (Lanctôt KL

Table 1: pharmacokinetic and pharmacodynamic characteristics of rivastigmine

Half life pharmacokinetic	1,5 hrs	Half life pharmacodynamic	10 h	T max	0,5- 2 h
Bioavailability	36%	Protein binding	40%	metabolism by CYP system	no
elimination	renal	dose starting	1,5 mg bid	dose maximum	6 mg bid
drug interaction	no	AChE G1	yes	AChE G4	yes

et al 2003). While the withdrawal rate due to adverse events was greater in the AChE inhibitor than placebo groups, the rate was in rivastigmine recipients 14% ; corresponding rates for the overall frequency of adverse events were 12%. Direct comparative studies of donepezil with rivastigmine (Wilkinson et al 2002) revealed tolerability findings similar to those of the above meta-analysis, and the double blind randomized comparative trial Exceed have been conducted (Bullock R et al 2005). More rivastigmine than donepezil treated patients reported 'any adverse event' during the 4- 14 weeks titration phase (82.0% and 64.7%, respectively). The higher rate of adverse events in the rivastigmine group during the titration phase appeared to be driven by an increased rate, relative to donepezil, of nausea (32.9% vs. 15.2%) and vomiting (27.9% vs. 5.8%) . In the maintenance phase weeks 17- 104, adverse event rates in the two groups were similar (78.7% for the rivastigmine group and 76.9% for the donepezil group). Premature discontinuations due to adverse events were higher in the rivastigmine group during the titration phase (14.1% vs. 7.0% for donepezil) but similar in the maintenance phase (17.9% vs. 14.1% for donepezil). There were no differences between rivastigmine and donepezil-treated patients with respect to number of serious adverse events (SAEs) and SAEs leading to discontinuation. Because the adverse effetcs are associated with peack plasma levels are in advanced development rivastigmine transdermal patches with better tolerability and equal bioavailability and efficacy.

Cholinesterases

With regard to cholinesterases, rivastigmine is the unic molecule that inhibits both AChE and butyrylcholinesterase (BuChE) from degrading Ach (Clegg et al 2001; Wolfson et al 2002), with an inhibition on G1 and G4 enzyme isoforms (Eskander et al 2005). AChE is located mainly in the neurons and BuChE in glial cells: BuChE action is more general and less understood than Ache activity mainly devoted to idrolize acetylcholine. BuChE activity is detected in all CNS areas receiving cholinergic innervation and although it represents only 10% of AChE activity in normal brain with the progression of the disease in AD brain BuChE increases by 40-90%, and contemporarely AChE decreases its activity by up to 45%. AChE and BuChE are present in several molecular isoforms, and in normal brain globular forms of four (G4) catalytic units are the most common, followed by one (G1). In AD brain the globular form G1 become predominant in the progression fo the disease as G4 levels declines, and several data suggests that BuChE action may be particulary relevant rather than AChE in subjects with moderate-severe stage of dementia. (Ach) (Tasker et al 2005).

Randomized Controlled Trials

A number of large, placebo-controlled, double-blind trials have demonstrated that cholinesterase inhibitor therapy results in significant improvements in cognitive, functional and global performances of patients with AD (Cummings et al 1996) , whilst preliminary evidence also indicates potential efficacy in the treatment of behavioural and psychiatric symptoms and disturbances (BPSD) (Minger et al 2000; Weinstock 1999; Giacobini 2000). Despite the evidence from the clinical studies and the intervening clinical experience the debate on whether ChEIs are effective continues.

Four studies controlled double blind vs. placebo for treatment duration ranged from 13 to 26 weeks have been conducted (Table 2). Participants included in all trials were classified as having probable AD of mild to moderate severity. All studies had three treatment arms, comparing various dosage levels of rivastigmine with placebo. Two trials (Corey-Bloom et al 1998; Rosler et al 1999) had treatment groups with doses of 1–4 and 6–12 mg/day (flexible-dose studies) and one trial had doses of 4 and 6 mg/day (Spiegel et al 1991). By the end of follow-up, the mean doses were similar for the two flexible-dose studies: 3.7 and 10.4 mg/day for the two groups in one (Rosler et al 1999) and 3.5 and 9.7 mg/day for the two groups in another (Corey-Bloom et al 1998). The remaining trial (Forette et al 1999) compared the effects of a twice-daily regimen compared with a three-times daily regimen, giving average doses of 9.6 and 10.1 mg/day, respectively. The trials were all multicentre studies, with total sample sizes ranging from 114 to 725 participants. The studies have demonstrated a statistically significant difference between drug and placebo at neuropsychological scales, clinician-rated global clinical state and activity of daily living. In the study by Corey-Bloom and colleagues (Corey-Bloom et al 1998) participants in the high-dose group showed an average decline that was 3.78 points less than the decline shown by placebo participants in the ADAS-Cog. The study reported in the MMSE a statistically significant difference between the high dose treatment group and placebo with an improvement in the high-dose group of 0.30 points, compared with a decline in placebo participants of –0.79 points. In the CIBIC- Plus the authors reported an average difference of 0.29 points between high-dose and placebo participants. In the GDS the high-dose groups' scores deteriorated by 0.19 points less than the placebo groups' scores. Finally in the PDS the study showed a statistically significant difference of 3.38 points between the 6–12 mg/day rivastigmine participants and the placebo group. The study by Agid and coll (Agid et al 1998) compared two fixed-dose groups (4 and 6 mg/day) with placebo, and did not report any statistically significant

Table 2. Randomized Controlled Trials of rivastigmine				
Study	Number of patients	Time/doses	Results	p-Value versus placebo
Corey-Bloom et al.,1998(36)	699 (centres: 22)	26 weeks 1-4 mg/day	ADAS-Cog 2.36 (3.13 to -1.59) MMSE - 0.34 CIBIC -plus 0.23 (0.07 to 0.39) GDS - 0.16 (-0.25 to -0.07) PDS 5.15 (-6.52 to -3.86)	
		26 weeks 6-11 mg/day	ADAS-Cog 0.31 (1.08 to -0.46) MMSE 0.30 CIBIC-plus 0.20 (0.04 to 0.36) GDS -0.13 (-0.22 to -0.04)	<0.001 < 0.05 <0.01 <0.03
		26 weeks placebo	PDS -1.52 (-2.85 to -0.19) ADAS-Cog 4.09 (4.86 to 3.32) MMSE - 0.79 CIBIC-plus 0.49 (0.33 to 0.65) GDS -0.32 (-0.41 to -0.23) PDS -4.90 (-6.22 to -3.58)	<0.001
Agid et al., 1998(xx)	402 (centres: 54)	13 weeks 4 mg/day	MMSE 0.0 ± 3.3 NOSGER (memory) 0.7 ± 2.8 NOSGER (IADL) 0.0 ± 3.3	
		13 weeks 6 mg/day	MMSE 0.0 ± 3.1 NOSGER (memory) 0.2 ± 2.4 NOSGER (IADL) -0.7 ± 3.5	Not reported
		13 weeks placebo	MMSE -0.0 ± 2.6 NOSGER (memory) 0.0 ± 3.4 NOSGER (IADL) -0.2 ± 3.3	

Forette et al., 1999 (38)	114 (centres: 11)	18 weeks twice/daily mean dose 9.6 mg/day 18 weeks three times/daily mean dose 10.1 mg/day 18 weeks placebo	ADAS-Cog - 2.6 NOSGER (memory) -0.7 ± 2.9 ADAS-Cog 0.41 NOSGER (memory) -1.0 ± 2.7 ADAS-Cog 2.0 NOSGER (memory) 1.3 ± 3.7	NS (0.054) 0.037 NS 0.014
Rosler et al., 1999 (37)	725 (centres: 22)	26 weeks 1-4 mg/day 26 weeks 6-11 mg/day 26 weeks placebo	ADAS-Cog 1.37 (2.27 to 0.53) MMSE -0.62 (-1.05 to -0.15) CIBIC-plus 4.24 (4.02 to 4.38) GDS -0.22 (-0.3 to -0.1 PDS -3.37 (-4.99 to -1.61) ADAS-Cog -0.26 (0.66 to – 1.06) MMSE 0.21 (-0.24 to 0.64) CIBIC-plus 3.91 (3.71 to 4.09) GDS -0.06 (-0.2 to -0.0) PDS 0.05 (-1.57 to 1.77) ADAS-Cog 1.34 (2.19 to 0.41) MMSE -0.47 (-0.96 to -0.04) CIBIC-plus 4.38 (4.22 to 4.58) GDS -0.26 (-0.4 to -0.2) PDS -2.18 (-3.91 to -0.49)	0.011 <0.05 <0.001 <0.05 0.07

differences between treatment groups and placebo for cognitive and functional outcome measure. In particular on NOSGER scale this study compared two different dose treatment groups with placebo. No p-values were reported for this outcome measure, but the high-dose rivastigmine group (6–12 mg/day) seemed to show an average improvement in memory and IADL performance (mean differences of –0.2 and –0.5, respectively). In the study designed by Forette and collegues (Forette et al 1999) patients taking rivastigmine b.i.d. improved more significantly in the CIBIC-Plus assessment of global functioning than those taking placebo. The size of treatment was large; 57% responders in the rivastigmine b.i.d. group vs. 16% in the placebo group. ADAS-cog scores also improved in patients receiving rivastigmine b.i.d. compared with placebo, but just failed to achieve statistical significance (p= 0.054). In addition, rivastigmine produced a significant improvement in the memory dimension of NOSGER. Although this study suggests an improvement in global function as rated by the physician (CIBIC-plus), function as assessed by psychometric tests (ADAS-Cog) and ADL as assessed by the carer (NOSGER), however, the sample sizes were very low (<30 participants in each group) and this study presented no information on power calculations. In the study by Rosler et al. (Rosler et al 1999) ADAScog improved in patients in

the higher dose group when compared with patients taking placebo (P < 0.05). Significantly more patients in the higher dose group had improved by 4 points or more than had improved in the placebo group (24% (57/242) v 16% (39/238)). Global function as rated by the CIBIC-plus scale had significantly improved among those in the higher dose group compared with those taking placebo (P < 0.001), and significantly more patients in the higher dose group showed improvement than did in the placebo group (37% (80/219) v 20% (46/230)). Mean scores on the progressive deterioration scale improved from baseline in patients in the higher dose group but fell in the placebo group. About GDS and MMSE, patients receiving placebo deteriorated by 0.47 points from baseline on the MMSE and those receiving rivastigmine 6-12 mg/day improved by 0.21 points over baseline using the intention to treat analysis. Significantly less deterioration occurred on the GDS among patients taking 6-12 mg/day rivastigmine compared with those taking placebo. In summary, statistically significant differences between the 6–12 mg/day treatment groups (mean dose ~10 mg/day) and placebo were reported by two of three published trials which reported ADAScog and MMSE. No statistically significant effects were seen in the low-dose treatment groups in these studies. Both (Corey-Bloom et al 1998; Rosler et al 1999) of the published studies which included CIBIC-plus as a global outcome measure reported a statistically significant improvement in high-dose participants (6–12 mg/day) compared with placebo participants. One study reported that a greater proportion of high-dose rivastigmine participants than placebo participants had a 'successful' CIBIC assessment, i.e. scoring 1 or 2 on the scale. The same trials (Corey-Bloom et al 1998; Rosler et al 1999) found a statistically significant improvement on the GDS measure in participants treated with 6–12 mg/day of rivastigmine compared with placebo participants. These studies reported the PDS (Progressive Deterioration Scale) as a functional outcome measure. One of these found a statistically significant improvement in participants treated with 6–12 mg/day rivastigmine compared with placebo, and the other reported that a statistically significantly higher percentage of these high-dose participants than placebo participants showed an improvement of at least 10%.

Head-to-Head Drug Comparisons

There are three randomized studies designed to compare two ChEIs, donepezil with rivastigmine. (Fuschillo et al 2001; Wilkinson et al 2002; Bullock et al 2005). In Fuschillo and colleagues' (Fuschillo et al 2001) study, a single-centre study of just 27 participants, those in the donepezil group were given 5 mg/day and those in the rivastigmine group had 1.5 mg/day for 1 week, increasing weekly in steps of 1.5 mg up to 6–9 mg/day, the duration of treatment was 30 weeks. In Wilkinson and colleagues'(Wilkinson et al 2002) study, those in the donepezil arm were given 5 mg/day for 28 days followed by 10 mg/day; those in the rivastigmine arm were initially given 1.5 mg twice daily for 14 days, then 3 mg twice daily for 14 days, then 4.5 mg twice daily for 14 days and finally, if tolerated, were given 6 mg twice daily. The study was a multicentre study (19 centres) with 112 participants open label, the participants knew which drug they were taking; the duration of treatment was 12 weeks. On measures of cognitive ability, both study suggests that treatment with rivastigmine (1.5–12 mg/day) leads to more improvement than treatment with 5 mg/day donepezil; however, these trends are small, are not tested for statistical significance and may also reflect the differences in the doses given. Rates of adverse events tended to be higher in those participants in the rivastigmine groups than the donepezil groups and more participants withdrew owing to adverse events in the rivastigmine groups. The effects of the doses reported may reflect on these differences. Recently, Bullock et al. (Bullock et al 2005) designed double blind, randomised, controlled, multicentre international trial to evaluate the efficacy and tolerability of cholinesterase inhibitor treatment in patients with moderate to moderately-severe Alzheimer's disease over a 2-year period. The number randomized was 994. The titration period was 16 weeks. The rivastigmine group started at 3 mg/day, and the dose was increased by 3 mg/day at 4 week intervals to a maximum of 12mg/day. The donepezil group received 5 mg/day in weeks 1-8 and10 mg/day thereafter. Following the 16 weeks titration patients were maintained at the highest tolerated dose level. The study showed that cholinesterase inhibitor treatment may offer continued therapeutic benefit for up to years in patients with moderate AD and, although both drugs performed similarly on cognition and behaviour, rivastigmine may provide greater benefit in activities of daily living and global functioning.

Long Term Studies

There is other evidence available from studies which are not randomized and double blind, the open label extension studies. These studies recruit patients who have been participating in a phase III randomized, double blind placebo controlled study to continue on open label treatment. (Table3)

Farlow et al. (Farlow et al 2000) reported the results of a 52-week 'delayedstart' rivastigmine study in mild-to-moderate AD. For the first 26 weeks, patients received placebo or rivastigmine. All patients were then eligible to

Table 3: Long term Studies of rivastigmine			
Study	Time	Study design	Objectives
Farlow et al (e)	1-year data	26-week open-label extension of a 26-week, placebo-controlled study (n = 533)	ADAS-cog: significant 5.7-point improvement compared with the projected placebo decline at 52 weeks (the end of the open-label extension
Grossberg et al (f)	2-year data	Meta-analysis of two open-label continuations of four placebo controlled studies, total duration 104 weeks (n =2010)	ADAS-cog: declined by 4–5 points less than predicted, had patients been left 'untreated
Small et al (g)	5-year data	Meta-analysis of two open-label continuations of four placebo controlled studies, maximum total duration 260 weeks (n = 2010)	ADAS-cog: mean annual decline of 3.9 points; patients remaining on rivastigmine for 5 years declined by about 20 points less than predicted for model-based 'untreated' patients MMSE: mean annual decline of 1.7 points; patients remaining on rivastigmine for 5 years declined by 7 points less than predicted for model-based 'untreated' patients

receive open-label rivastigmine for another 26 weeks. There was a significant treatment difference of 5.7 points with rivastigmine on ADAS-cog for patients remaining on rivastigmine for 52 weeks ($p < 0.001$), compared with the projected decline if they had been left 'untreated', calculated by using a statistical model. In addition, patients who received placebo for the first 26 weeks and were switched to rivastigmine for weeks 27–52 did not 'catch up' with those who were on rivastigmine from the beginning of the trial (1.4-point difference on ADAS-cog).

The effects of rivastigmine on cognition were showed to persist for up to 2 years in a meta¬analysis of 2010 patients with AD taking part in four 26-week, placebo-controlled studies followed by open-label extensions (Grossberg et al 2004). Patients remaining on rivastigmine for up to 2 years showed 4–5 points less decline on ADAS-cog, compared with projected decline if they had been left 'untreated'. These conclusions are based on a comparison of the actual clinical changes measured in patients treated with rivastigmine in open-label studies, with hypothetical clinical changes derived by predicting the scores of those same patients had they been untreated, using a baseline-dependent model derived from data in an untreated AD population (Grossberg et al 2004).

Most recently, this meta-analysis (Grossberg et al 2004) was 'updated' with patients remaining on treatment for up to 5 years (Small et al 2005). These data provided the

longest-term efficacy data for any ChE-I to date. Even though only 83 patients remained under study conditions at 5 years, these data can be considered informative, because most patients tend to discontinue ChEI treatment over time (Bullock et al 2005). Mean baseline MMSE and ADAS-cog scores at entry into the placebo-controlled studies were 19.3 and 24.6, respectively. Mean MMSE and ADAS-cog scores of patients remaining on rivastigmine for 5 years were 12.7 and 36.8 (both showing 'moderate' AD) (Small et al 2005). Patients remaining on rivastigmine for 5 years declined on average by 1.7 points each year on the MMSE, or 3.9 points each year on ADAScog. These cognitive declines were smaller than those predicted using baseline-dependent models of 'untreated' patients, and smaller than those reported for untreated patients in the literature (Bullock et al 2005) .

Systematic Reviews

Several reviews on the treatment of AD and in particular on rivastigmine have been published in the last years. We reported the most relevant sistematic reviews, particularly reliable because at the standardized methodology used to select the studies to be analyzed.

The Health Technology Assessment (HTA) (Loveman et al 2006) Programme recently published a study to provide an update review of the best quality evidence for the clinical effectiveness and cost-effectiveness of donepezil, rivastigmine and galantamine for mild to moderately severe Alzheimer's disease and of memantine for moderately severe to severe AD. The authors concluded that there is evidence from studies using cognitive and global outcome measurement scales that rivastigmine may be beneficial in AD and that this is particularly so at higher doses (6–12 mg). On global measures, benefit was similarly demonstrated with the higher doses of rivastigmine only. Rivastigmine also appears to be beneficial at higher doses on measures of function, although this was not always demonstrated with statistical significance. Adverse events were more common with higher doses of rivastigmine, although nausea and vomiting were associated with treatment in general.

The Cochrane Collaboration (Birks et al 2002) examined published works to 2000 to determine the clinical efficacy and safety of rivastigmine for patients with AD. Seven trials were identified which met the criteria for inclusion in this review, involving 3370 participants. Use of rivastigmine in high doses (6 to 12 mg daily) was associated with a 2.1 point improvement in cognitive function on the ADAS-cog score compared with placebo and a 2.2 point improvement in activities daily living assessed on the Progressive Deterioration Scale at 26 weeks. At lower doses (4 mg daily or lower) differences were in the same direction but were statistically significant only for cognitive function. Significant differences in the CIBIC-Plus were seen at 26 weeks but not earlier. Besides, most recently the Cochrane Collaboration (Birks 2006) assessed the effects of donepezil, galantamine and rivastigmine in people with mild, moderate or severe dementia due to Alzheimer's disease. The results of 10 randomized, double blind, placebo controlled trials demonstrate that treatment for 6 months, with donepezil, galantamine or rivastigmine at the recommended dose for people with AD produced improvements in cognitive function, on average 2.7 points (95%C -3.0 to -2.3, p.0.00001), in the midrange of the 70 point ADAS-Cog Scale. Study clinicians rated global clinical state more positively in treated patients. Benefits of treatment were also seen on measures of activities of daily living and behaviour. The effects are similar for patients with severe dementia, although there is very little evidence, from only two trials.

Conclusions

Since the discovery of a substantial pre-synaptic cholinergic deficit in AD brains 25 years ago, a large body of experimental data has emerged examining the nature, extent and clinical significance of this change. Several studies have shown in AD various abnormalities of many neurotransmitter systems (particularly glutamatergic changes), the most prominent of which is the severe damage of the cholinergic system with a selective loss of presynaptic cholinergic neurones projecting to cerebral cortex and hippocampus, leading to the so-called "cholinergic Hypothesis". Some of the symptoms of AD are then thought to be due to cholinergic deficit, and this theory has led to several therapeutic approaches attempts to restore cholinergic activity in the CNS. To date, the most successful approach involves ChEI which increase the amount of acetylcholine at the neuronal synaptic cleft by inhibiting the enzyme responsible for its degradation, thus improving neuronal transmission; the more recent molecules are selective, acting at central level, minimising side effects. In AD patients brain there is a loss of glutamatergic pyramidal neurons, while the number of glutamate receptors is maintained (in particular N-metil-D-aspartate, NMDA, receptor). Based on these evidences, a therapeutic use of glutamatergic-blocking molecules has been proposed. Memantine, an N-metil-D-aspartate, NMDA blocker, has had positive results reported in severe Alzheimer's disease (Wilkinson et al 2002) and in association with rivastigmine (Dantoine et al 2006) and other ChEi molecules. Cholinesterase inhibitors and Memantine are the only FDA-approved pharmacologic treatments for AD.

The guide lines for clinical practice (Doody et al 2001;

Davies et al 1976; Caltagirone et al 2005) have derived treatment indications from RCTs. Many problems are still unsolved with regards to the transfer of information from an experimental setting to clinical practice (Scneider 2006). A limitation of RCTs on AD is the long duration of disease (years) facing the short duration of clinical trials (weeks); they could then fail in supplying long term information on treatment effects.

Moreover, RCTs' end-points estimates are surrogate endpoints (e.g. the ADAS Cog cognitive improvement to 3 - 6 months) considered as valid substitutes of real end points (g.e. to stabilise or totally improve functions of the affected subject in the long term). Moreover, usual doubts about enrolment/exclusion criteria from clinical trials arise: to select a population much too different from clinical practice, particularly in terms of comorbility and multi-therapy.

The efficacy of medical interventions is their capacity of inducing positive modifications of the natural history of diseases. The natural history of dementia is marked by specific events related to the cognitive and functional decline, but their occurrence is poorly predictable in individual patients being highly variable from patient to patient. For this reason it is difficult that the modest efficacy of available interventions for dementia, or their entity measured by clinical scales, may be perceived in clinical practice or in observational studies Moreover in randomized clinical studies, the effect of this variability, in analogy to misclassification of exposition and/or disease in case control or cohort epidemiological studies, is that of an underestimation of the true efficacy of interventions.

Rivastigmine has been shown to be effective in the treatment of the cognitive, behavioural, and functional deficits of AD (Birks et al 2002). The RCT studies have demonstrated a statistically significant difference between drug and placebo at neuropsychological scales, clinician-rated global clinical state and activity of daily living.

The benefits from this drug however are limited and its long term effectiveness has not been well-demonstrate. Research is required to generate relevant data on long-term outcomes, disease progression through relevant health states, quality of life and costs of treating people with Alzheimer's disease with rivastigmine.

In fact, as Alzheimer's disease generally progresses slowly and a clinical course of 5 or 10 years is not unusual, clinical trials of 6 or 12 month duration of treatment are of limited use.

Unfortunately, randomized trial evidence of longer term effects is not currently available and given the widely differing rates of progression of Alzheimer's disease in different individuals and groups selected in different ways, extrapolation could be misleading. There are reports of open-label extensions to some of the included studies. Data suggest that patients remaining on rivastigmine for up to five years showed as smaller decline on cognitive aspects compared with projected decline if they dad been left untreated. The results of open-label extension trials should be interpreted with caution. In fact, there are several reasons for possible bias; not all patients enter the extensions to the trials, only a self selected group, the comparisons are made with historical controls, or with a hypothetical placebo decline obtained by extrapolation from the randomized phase. There is a need for randomized, placebo controlled trials of longer than one year; at the moment, in fact, only one study is from 5 years.

Another unsolved and frequent problem is the decision about the duration of treatment and criteria of suspension, and other studies will be needed to help establish the maximum duration of treatment, and the indicators that could show when treatment is no longer beneficial.

Nevertheless, new data suggest that cholinesterase inhibition may provide for up to 5 years. It is important for patients and caregivers to understand that they should not expect increasing improvements over the long term, but rather the aim is to maintain the patient's status at a manageable level, and for patients to continue to be themselves.

At moment, head-to-head trials of rivastigmine vs donepezil are limited, so that no guide lines for clinical treatment can be derived, existing trials suggest no major differences in efficacy (Bullock et al 2005). Both drugs performed in the same way on cognition and behaviour, rivastigmine may provide an improvement in ADL and global functioning but there were some differences in the doses given (Wilkinson et al 2002).

A recent published open-label study sought to evaluate the efficacy of the ChE inhibitor rivastigmine on cognition, functional autonomy and behavior in patients with mild-to-moderate AD previously treated with other ChE inhibitors (switched patients). The authors conclude that the patients switched from previous ChE inhibitor therapy to rivastigmine can obtain measurable benefits, although the treatment effect may be less than in de novo patients (Gauthier 2006).

Finally, the published guidelines are by the National Institute for Clinical Excellence (The NICE 2001) (www.nice.org.uk) that appraised these drugs in 2000 and endorsed their use when a number of conditions were met. Treatment guidelines recommend that ChE-I treatment should be continued only if there is an increase, or no decrease in MMSE score 2-4 months after reaching the suitable dose. At the time there were some gaps in the evidence base; in particular the impact on QoL and the evidence on cost-effectiveness were lacking which resulted in some uncertainty about the guidance provided. The evidence base has since advanced and NICE are currently reviewing their original appraisal.

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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. 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

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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).

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)

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

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 2. Baseline characteristics by follow-up status.

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

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

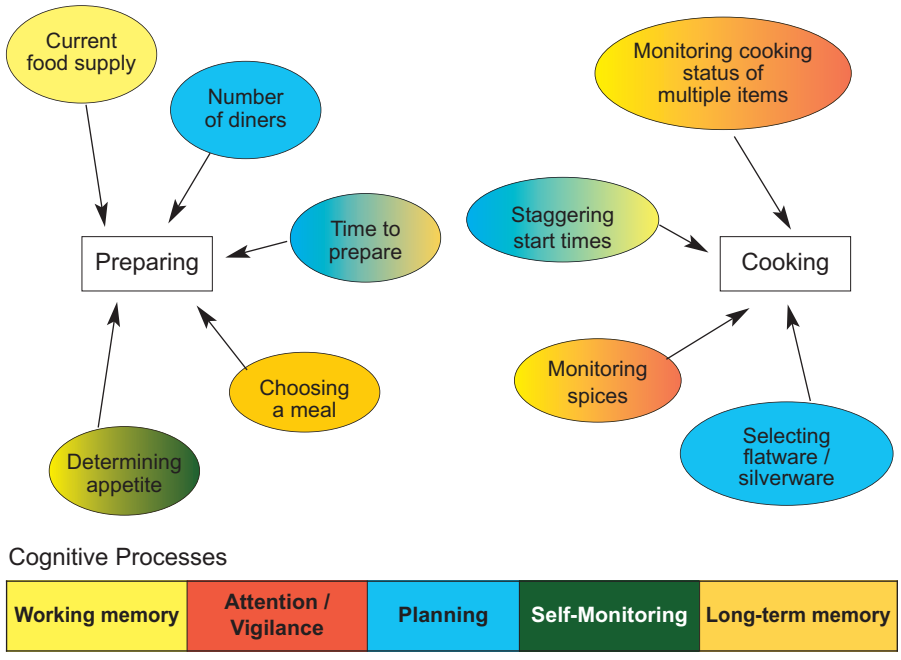
baseline versus 'no impairment in *Food Preparation*' (Table 5). People with any impairment in *Food Preparation* at baseline were significantly more likely to progress to AD than those with no impairment (Odds Ratio = 8.33, Confidence Interval = 1.03 – 67.14).

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 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.

Figure 1. Cognitive processes associated with food preparation



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

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 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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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 hispathology, Jeong et al. (2002)[5] suggested that MuLV could play an 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

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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 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

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.

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

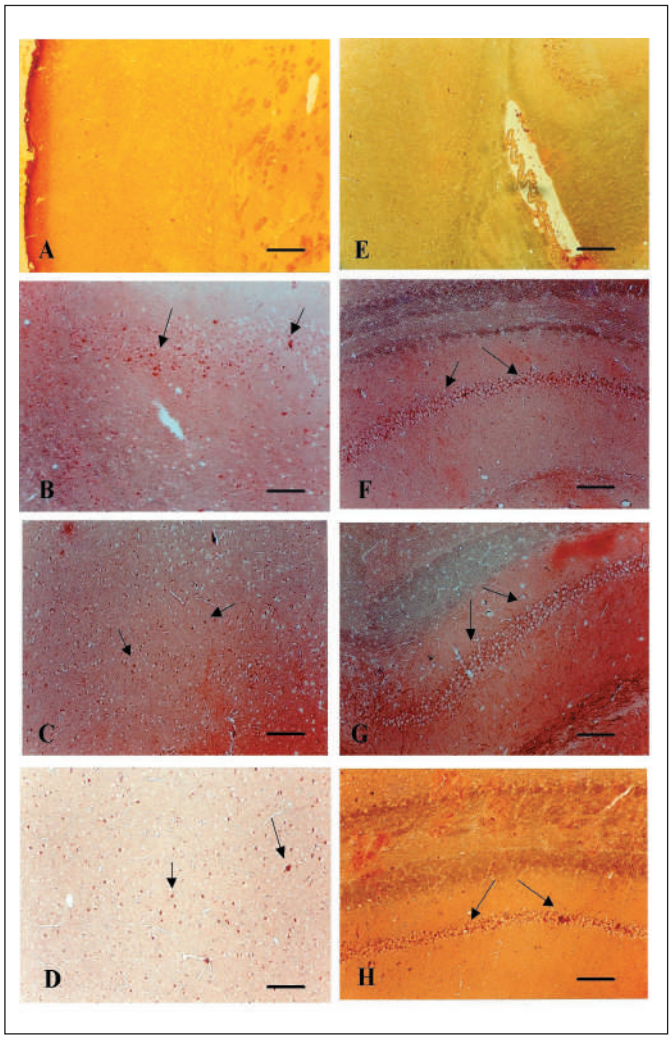

Figure 1.
A. Very few and weak HNE-immunostaining neurons were found in the cortex (layer I to layer VI) of SAMR1 mice. Bar: 200µm.
B. Increased number and intensity of HNE-immunostaining neurons (arrows) in the cortex (frontoparietal cortex, layer II, motor area) of SAMP8 mice. Bar: 100µm.
C. Increased 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.

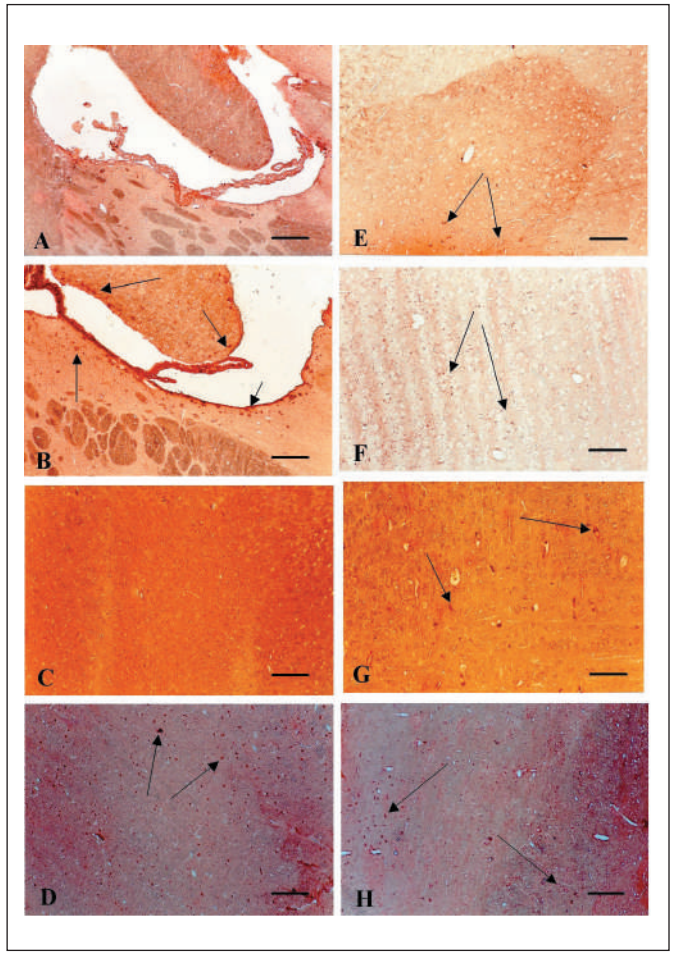

Figure 2.
A. Very few and weak HNE-immunostaining neurons were found in the fimbria hippocampus, stria terminalis and stratum subependymale ventriculi lateralis of SAMR1 mice. Bar: 200µm.
B. Increased number and intensity of HNE-immunostaining neurons (arrows) were found in the fimbria hippocampus, stria 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.

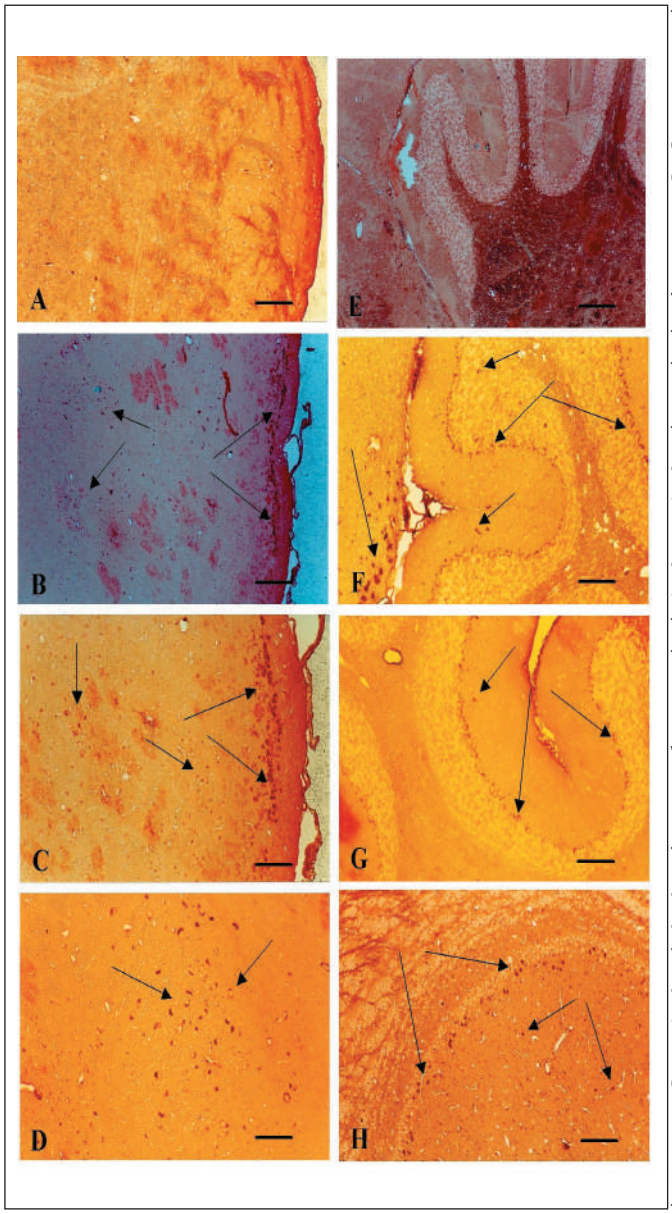

Figure 3.

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.

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).

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 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 hy-

pothesis 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 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 ox-

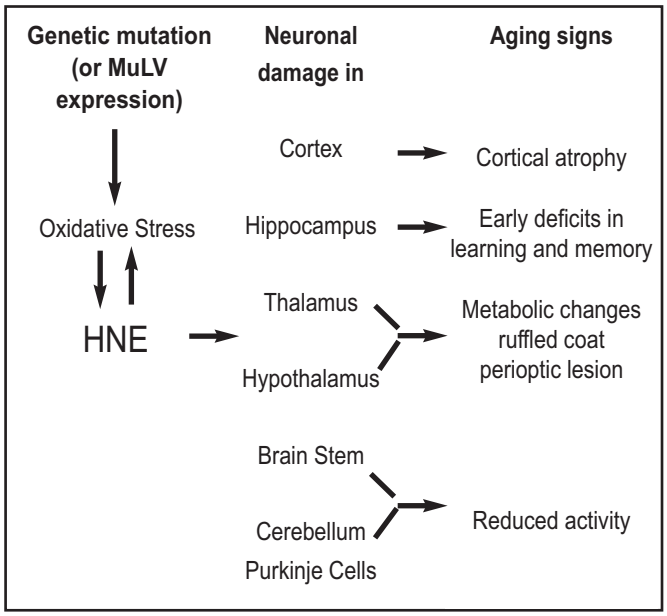

Figure 4. Possible Results of Oxidative Stress and Brain Damage in SAMP8 and SAMP10 Mice.

idative 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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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 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

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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

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.

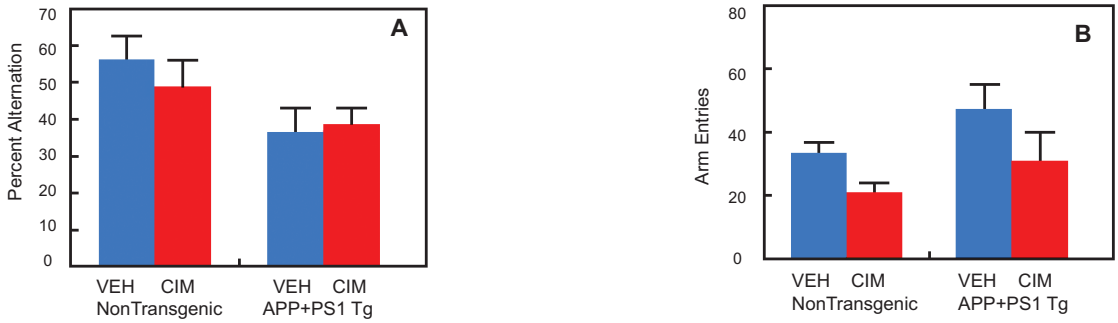
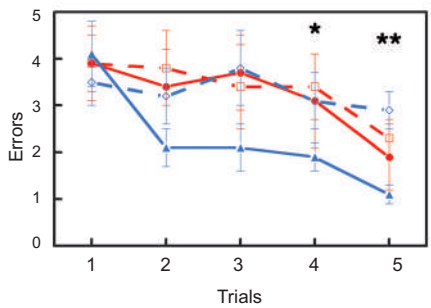


Figure 2. Radial arm water maze performance in transgenic and nontransgenic mice treated with cimetidine or vehicle. Mice were tested in a radial arm water maze. Data presented are the average of days 8 and 10 (day 9 day had to be discarded; see methods). Incorrect arm entries are presented as errors. 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).



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).

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

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.

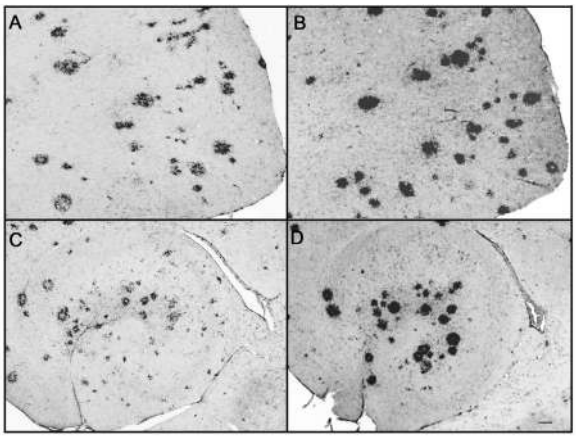
Table 2. Amyloid and GFAP loads in transgenic mice treated with cimetidine or vehicle.

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

of cimetidine on Congo red staining, or on Thioflavine-S staining (table 2).

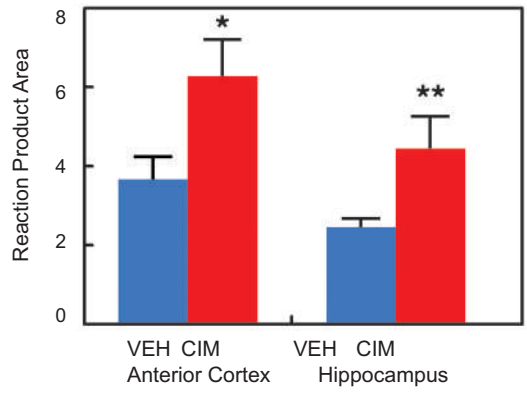
Cimetidine caused a significant activation of microglia in the APP+PS1 mice. A micrograph demonstrating this effect for the microglia marker CD45 is shown in Figure 3, where the microglial staining seen in vehicle treated mice (panels 3A, C) is lower than that found in mice treated

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.



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 anti-

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. * $P < 0.05$; ** $P < 0.01$.



gen 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).

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.

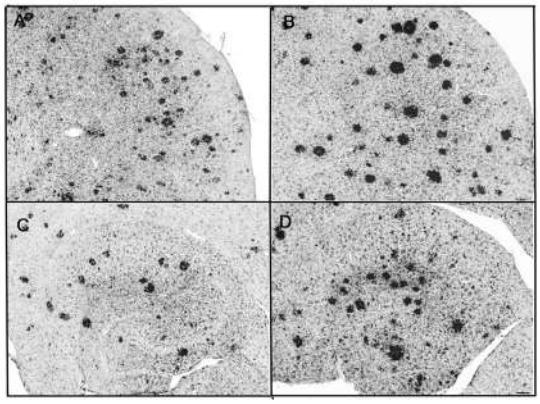
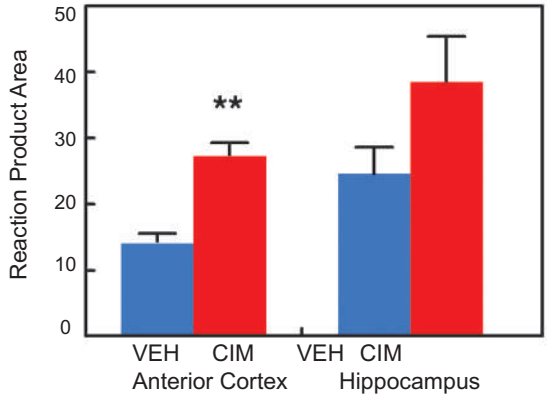


Figure 6. Cimetidine results in increased CD11b immunohistochemistry in anterior cortex following 12 months of cimetidine treatment via drinking water. Data are expressed as the percentage of the measured area that is positively stained by immunohistochemical reaction product. "VEH" (blue bars) are vehicle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



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 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 anti-histamine 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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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 [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

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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.

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 biop-

sies 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

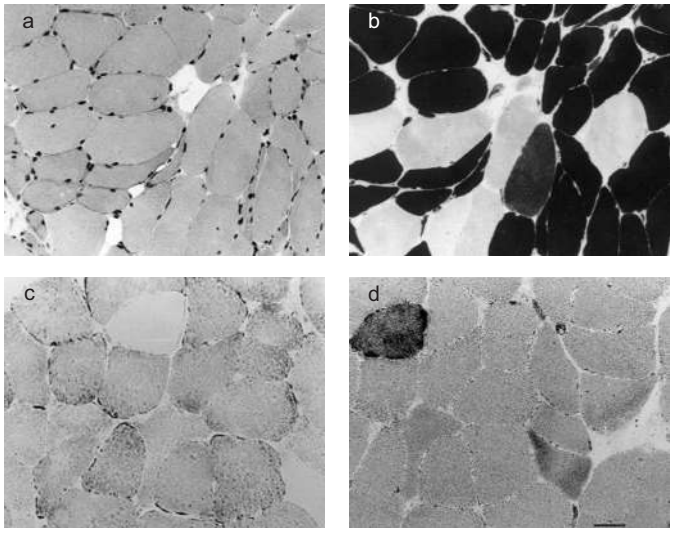

Figure 1. Muscle biopsy from m. tibialis anterior of a patient with Alzheimer's disease. Cross-sections were stained with Htx-eosin (a), for myofibrillar ATPase after acid preincubation at pH 4.6 (b), and for COX (c) and SDH (d). In (a) and (b), there are small groups of angulated atrophic fibres of 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.

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 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

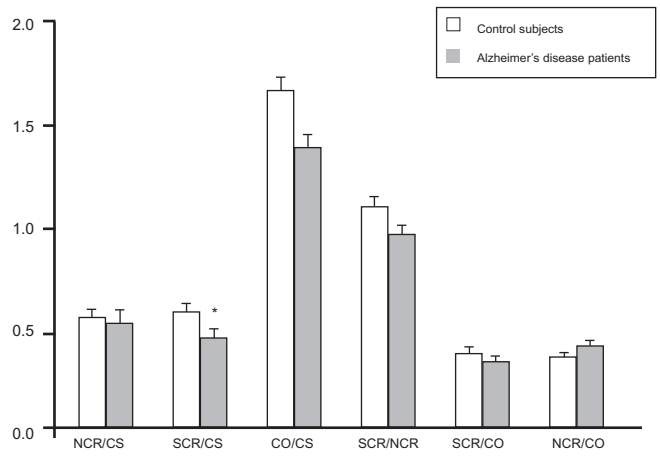

Figure 2. Enzyme activities in isolated mitochondria from muscle tissue. All enzyme activities were measured as mmol/min/L mitochondrial suspension. Data are means ± SEM, n=10 (Alzheimer's disease patients) or 9 (control subjects). *p<0.05, Mann-Whitney's U-test.

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.

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.

Acknowledgements

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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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Standardized Geriatric Evaluation in a Group of Patients with Thyroid Pathology and Dementia of Alzheimer's Type.

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Abstract

Actually, Alzheimer's disease (AD) is the most important neurodegenerative illness. Its prevalence is growing by 0.2% to people between 55 and 64 and by 27% to those of 65 and beyond. The first symptom of the disease is the cognitive deficit, that progressively worsens over the time. Although thyroid pathology is not a risk factor for AD, its early diagnosis and prompt treatment could help the prognostic of the cognitive deficit's future evolution. The evaluation of the cognitive deficit in an AD patient only based on psychometric tests, without the exclusion of thyroid pathology, could be erroneous. Our study was realized at the Geriatric and Gerontology Clinic, Elias Hospital – Bucharest, during March 8, 2004 – May 31, 2005, where the standardized geriatric evaluation was performed for each of the 850 patients enrolled in the study. From those, 327 (38,5%) was registered as having dementia, while 41 as presenting associated thyroid pathology.
Keywords: dementia, cognitive deficit, standardized geriatric evaluation

Early diagnosis of the dementia is actually extremely important due to the growing number of the afflicted elderly, and consecutively to the big social and economical costs annually allocated from health budgets. During March 8, 2004 – May 31, 2005, a number of 850 patients were warded at the Geriatric and Gerontology Clinic of the "Elias" Emergency Hospital from Bucharest. At the ending of the evaluation, 327 (38,5%) of them were diagnosed as having deferent dementia types, as shown in fig.1. The distribution of dementia types' reveals the net prevalence of Alzheimer's (degenerative) pathological entity in 254 (77.7%) of the patients, followed by mixed dementia in 55 (16.8%) of them and vascular dementia in the rest of 18 (5.5%).

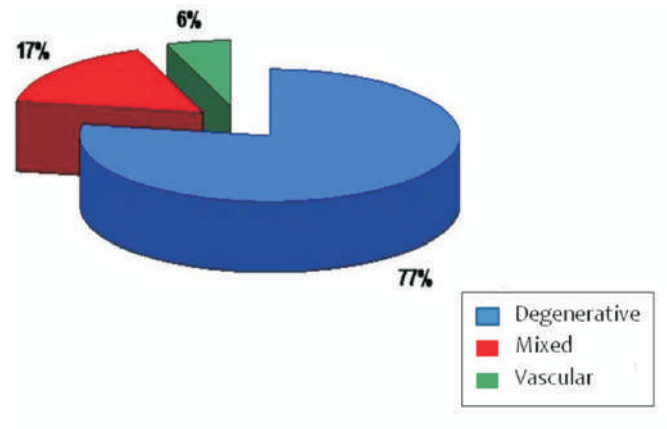


Fig.1 Distribution of the dementia types among the patients warded during the study.

Associated thyroid pathology was identified in a number of 41 (4.8%) patients from the entire patients group. In 10 of them the clinic and paraclinic exam excluded the diagnostic of dementia, but revealed instead a mild cognitive deficit (MMSE score among 25–30), accompanied by anxious-depressive disturbances in 6 patients and depression in one patient. The fact that the mild cognitive deficit that appears in anxious-depressive states and thyroid diseases is understood as differing from that involved in the conversion from normal aging to dementia (mostly of Alzheimer's type), and requires a different diagnosis algorithm, these 10 patients were kept under medical survey but removed from the study. So, the final number of patients with dementia and associated thyroid pathology kept under study was 31.

The individual scores obtained from the cognitive and behavioral assessment tests were analyzed in accordance with the specific directions of each test. A qualitative approach of the quantitative values was done, so that a MMSE score between 28 and 30 for example indicates a mild cognitive deficit.

Standardized Geriatric Evaluation (SGE)

1. The evaluation of the cognitive deficit according to Mini Mental Status Evaluation (MMSE).

The MMSE score has been assessed to 30 out of the 31 patients, because in one patient it failed to be performed. In accordance with the diagnostics criteria of our Clinic, a test is considered failed in the case of a patient with severe cognitive deterioration that means a MMSE score lower than 10.

According to the obtained data, mild cognitive deficit was

detected in 20 (66.8%) patients for which were registered scores between 25 and 30 (Table nr.1). Mild to moderate cognitive deficit (score between 20 and 24) was detected in 6 (20%) patients, while 2 (6.6%) patients exhibited moderate cognitive deficit (score between 15 and 19). In other 2 patients (6.6%) a moderate to severe cognitive deficit (MMSE scores among 10 - 14 were detected).

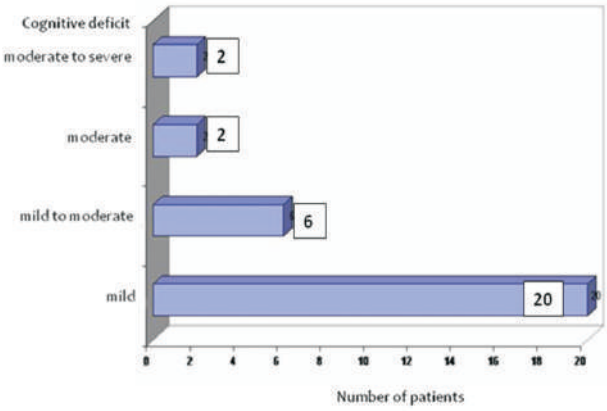


Fig. 2 – Distribution of cognitive deficit types in the patients under study, in accordance with MMSE scores

The indicators related to MMSE scores distribution are as in the table nr.1.

Table No. 1 - Indicators of MMSE scores distribution

Indicator	Value
Mean score	25.5
Median score	28
Modal score	29
Interval of variation	13 – 30
Standard deviation (sx)	± 5.0
Coeficient of variation	19.6% (median dispersion)

2. The horologe test (clock test)

Clock test was applied to 22 patients (71.0%). Eight patients (36.4%) achieved a score of 10, 4 (18.2%) a score of 7, and 3 (13.6%) a score of 8 (Table nr.2). A null score has been registered in 4 patients (18.2%) and a score of 1 in 2 patients (9.1%). A single patient out of those to whom horologe test was applied achieved a score of 6 (fig.3).

The indicators of Clock Text scores distribution are shown in table nr. 2.

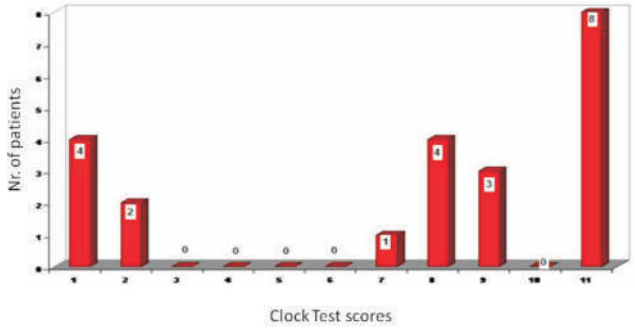


Fig. 3 – Patients distribution depending on Clock Test scores

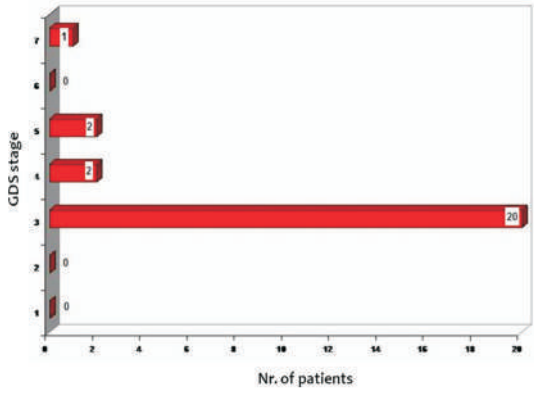
Table No. 2 - Indicators of Clock Test scores distribution

Indicator	Valoare
Mean score	6.4
Median score	7.5
Modal score	10
Variation interval	0 - 10
Standard deviation (sx)	± 4.0
Variation coeficient	62.5% (large dispersion)

3. Global Deterioration Scale – GDS

Out of the 25 patients to whom was applied the GDS, 20 (80.0%) have fallen into the 3rd scale stage – "mild cognitive deficit", 4 (16.0%) in stages 4 and 5 – "mild–moderate to severe cognitive deficit". Only 1 patient has fallen into the 7th GDS scale stage – "few apparent cognitive functions". It was the patient in whose case the MMSE assessment failed to be performed (fig.4).

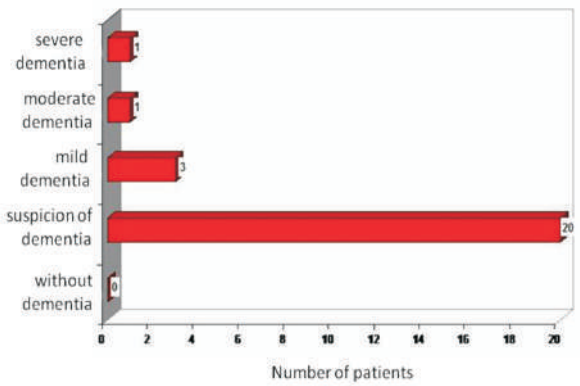
Fig. 4 – Patients distribution depending on their global deterioration stage.



4. Clinical Dementia Rating scale – CDR

The distribution of the 25 patients whose cognitive evaluation was performed according to the CDR scale presented, as expected, similar aspects with those obtained through the global deterioration scale (GDS). Thus the score of 0.5, which indicates "the suspicion of dementia" was registered in 20 patients, while score 1 - "mild dementia" and the score 2 - "moderate dementia" was detected in 4 patients. The patient in which MMSE failed to be applied has fallen in the 5th stage of the scale – "severe dementia" (fig.5).

Fig. 5 – Patients distribution depending on the stage appreciated by means of the CDR scale.



The indicators of SCD scores distribution are shown below, in table nr.3.

Table Nr. 3 - indicators of SCD scores distribution.

Indicator	Valoare
Mean score	0.7
Median score	0.5
Modal score	0.5
Variation interval	0.5 - 3
Standard deviation (sx)	± 0.6
Variation coeficient	85.7% (large dispersion)

5. Nutritional evaluation – The Mini Nutritional Assessment test (MNA)

The scores obtained in all of the 31 patients are higher than 23.5, indicating a good nutritional state.

6. The evaluation of the static and dynamic equilibrium – Tinetti test

Except from 1 patient, in which obtained scores indicated difficulties in maintaining the static equilibrium (Tinetti Static test score 39) and the dynamic equilibrium (Tinetti Dynamic test score 18), in all of the other the values have maintained in-between the normal limits.

7. Norton Score for the trophic lesions

Except 1 patient in which the score was 13 (risk of trophic lesions), a score greater than 14 (absence of trophic lesions risk) was registered in the other 30 patients.

Taking into account the above results, we consider that they plead for the use of Standardized Geriatric Evaluation as a powerful tool for the early diagnosis of cognitive deficit in patients with associated Alzheimer's dementia and thyroid pathology.

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Görel Bråne Scale (GBS)

Introduction

The Gottfries-Brane-Steen (GBS) scale is a rating scale measuring three types of function (intellectual, emotional and activities of daily living [ADL]) and some behavioral and psychological symptoms common in dementia. The scale is well established and has been in use for more than two decades. This supplement presents a number of translations into different European languages.

The percentage of elderly people is increasing rapidly throughout the world, which consequently also leads to an increase in the number of demented people. In most countries, considerable amounts of money are allotted to care for people with dementia and more will be needed in the near future. Research into dementia is going on all over the world and therapeutic strategies (pharmacological and psychological) are tested in clinical trials. Some of the reports are encouraging. There are responders among the demented, i.e., people who improve or do not deteriorate during the test periods. To be able to compare results across countries, every participating country needs sensitive rating scales that measure relevant symptoms in a holistic way and that are written in the language or languages spoken in that country.

Rating scales for dementia are also needed to provide a common language (within the official one) for different staff groups. Well-formulated and adequate descriptions of symptoms, as well as well-defined scale steps, are helpful in reducing subjectivity and increasing knowledge and understanding. The use of rating scales has proven important in education and supervision of staff. Rating scales are also important in monitoring the effects of new drugs for dementia.

Most countries are today multi-cultural, with immigrants from many parts of the world. There is a group of demented people who have a poor knowledge of the language spoken in the country where they live. There are also relatives and staff members among the immigrants. Rating instruments with text in different languages may facilitate communication in these situations.

The Gottfries-Brane-Steen (GBS) scale is a concrete Swedish rating scale for evaluating dementia syndromes. It can be used by different types of professionals and it is easy to use after a short introduction/training.

It is a comprehensive semi-structured observer rating scale. An evaluation is made from an interview with the patient and observations of the patient during the interview. Information from a caregiver or key person may also be needed. The scale includes 27 items and has three subscales, the intellectual (12 items), the emotional (3 items), and the activities of daily living (ADL; 6 items) scales. It also has a section for rating of some behavioral and psychological symptoms that are common in dementia (6 items). Every item has 7 scale steps, of which 0,2,4 and 6 are defined. 0 means absence of impairment or symptoms and 6 means maximal impairment or maximal severity of the symptom.

For each item, 0 indicates normality, 1-2 indicates mild impairment, 3-4 moderate impairment and 5-6 severe impairment or, in the case of the symptoms, 0 indicates absence of the symptom, 1-2 indicates that the symptom is mild, 3-4 that it is moderate and 5-6 that it is severe. The scores from the assessment are transferred to a "GBS profile", which may be used to obtain a general overview of the degree of dementia in individual patients or in specific patient groups.

The dementia conditions constantly produce changing patterns of disabilities and symptoms at different stages. Nowadays it is possible to treat both emotional and cognitive symptoms pharmacologically. The GBS scale has been used as an evaluation instrument in many such studies.

Different languages

As far as we know, the GBS-scale has today been translated into about 20 different languages, but we suppose there might be translations that are unknown to us.

The procedure normally followed when producing a version of the GBS scale in a new language is to translate the scale on the basis of the English version and then have it "back translated" into Swedish to check that the translated item descriptions agree with the original ones. Among the translated versions of the GBS scale, several have been back translated, several have been studied for their reliability and been published in national journals. Some of the translated versions include a language item, i.e., an item added to the revised English version. The aim of this work was to present translations of the GBS scale into European languages.

The GBS scale for assessment of dementia

Recommendations:

GBS ratings should be made by physician, nurses, psychologists or social workers.
Rater training in advance is important.

A GBS rating should be based on observation of the patient. It can be made during or after an interview that lasts approximately 20 minutes. This should be semistructured, i.e., the patient should be given opportunity to tell the rater freely about his/her problems, but the rater should also give the patient structured questions. It may be necessary to get complementary information from a caregiver or key person.

Reference

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Instructions

Assess the patient's condition on the basis of the descriptions in this form. For each item, tick the relevant defined alternative (0, 2, 4 or 6) or in between the two most fitting alternatives (1, 3 or 5).

Intellectual impairment

	0	1 2	3 4	5 6
Orientation to person	Knows his/her name, occupation, age and date of birth. ☐	Knows his/her name but may have a deficient knowledge of his/her occupation, age and date of birth. ☐ ☐	Remembers his/her name passably but not his/her occupation, age or date of birth. ☐ ☐	Completely disorientated to person. ☐ ☐
Orientation to time	Completely oriented to time; knows the day of the week, date, month and year. ☐	Partly oriented to time; knows at least two of the time variables year, month, day of the week and date. ☐ ☐	Knows which season it is but not the day of the week, date, month or year. ☐ ☐	Completely disorientated to time. ☐ ☐
Orientation to space	Knows which geographical place, e.g. hospital, department, ward or room, he/she is in. ☐	Has some deficits in spatial orientation but finds the way in his/her own ward or home. ☐ ☐	Disorientated, i.e. has difficulty in finding the way in his/her own ward or home. ☐ ☐	Completely disorientated to space. ☐ ☐
Recent memory	No disturbance of recent memory; knows what has happened in the last few days. ☐	Recent memory is impaired, but this is evident only in exhaustive conversation. ☐ ☐	Such severe impairment of recent memory that it is evident in easy conversation. ☐ ☐	Recent memory completely lost; cannot remember anything from moment to moment. ☐ ☐

Distant memory	No disturbance of distant memory; remembers in exhaustive conversation the names of persons who have been significant to him/her and important political and/orother events from earlierperiods of his/her life.	Difficulty in remembering persons who have been significant to him/her and important political and/or other events from earlier periods of his/her life.	Such severe impairment that it is evident in easy conversation, e.g. cannot recall the names of the family members nor remember how many they are or where they live.	Distant memory completely lost.
Wakefulness	Fully awake.	Occasionally appears slightly drowsy.	Shows signs of drowsiness but can be kept awake with slight encouragement.	Somnolent; i.e. drowsy; can be awakened but soon relapses into drowsiness.
Concentration	No difficulty in concentrating; no difficulty in collecting his/her thoughts in the interview situation, follow a TV programme and/or read a text.	Occasionally appears to lose concentration, i.e. digresses and has some difficulty in reading or following a TV programme.	Markedly disturbed concentration; difficulty in keeping to the subject or finding coherence in TV programmes, news paper articles, etc.	Power of concentration so severely impaired that meaningful conversation is impossible.
Ability to increase tempo	Can hurry when required.	Manages to do what is required even if he/she is rushed, but then performance is markedly impaired.	When he/she is rushed performance is so severely impaired that not even simple tasks can be carried out and he/she becomes irritable and/or confused.	Reactions so blunted th<he/she does not react at all when being rushed.
Absent-mindedness	Normally collected.	Occasionally appear absent-minded.	Moderately but constantly absent-minded.	Constantly very absent-minded; incapable of keeping him/herself purposefully occupied.
Long-windedness	Expresses him/her self normally; is not wordy and can keep to the subject.	Occasionally wordy. using overdetailed descriptions but has no difficulty at all in keeping to the subject.	Is constantly wordy and reports unending details; has difficulty in coming to the point and frequently digresses.	Incapable of expressing what he/she wants and gets completely lost in wordy details.

Distractibility	Is able to maintain attention; is not distracted by irrelevant stimuli.	Attention occasionally distracted by irrelevant stimuli.	Attention markedly and constantly distracted by irrelevant stimuli.	So severely distracted b) irrelevant stimuli that meaningful activity (e.g. easy tasks or ordinary conversation) is impossible.
Language disturbances	No language disturbances, speaks faultlessly and fluently and understands what is said to him/her.	Occasional language disturbances, e.g. in the form of a limited vocabulary and slow, jerky, but fully understandable speech or speech that is fluent but difficult to understand or in the form of confusion of sounds/ words or reduced ability to understand other people's speech.	Frequent language disturbances in ordinary conversation, e.g. a very limited vocabulary and very slow but understandable speech or repeated confusion of sounds/ words, fluent nonsense talk, or substantially reduced ability lo understand other people's speech.	Cannot make him/herself understood verbally. Has constantly great difficulty in understanding other people's speech.

Emotional impairment

	0	1 2	3 4	5 6
Emotional functions	No disturbance of Emotional functions; is able to react with sorrow, joy, hate, fear anger, etc. in different situations.	Occasional disturbance with signs of sorrow, joy etc., but the subtle nuances previously characteristic of the person are lost.	Occasionally shows signs of sorrow, joy, etc. but in a coarse and unvaried manner.	Emotional functions completely lost; incapable of showing signs of sorrow, joy, etc.
Emotional liability	Is able to control Emotional reactions.	Weeps or laughs in an uninhibited or exaggerated manner on strong emotional stimulation.	Reacts in an uninhibited manner on moderate emotional stimulation.	The ability to control emotional reactions is completely lost.
Motivation	Normal motivation for activities.	Requires considerable encouragement to start an activity and usually shows only a passive interest in it.	Clearly lacking in motivation; requires continual firm direction to start an activity or complete a task.	Not motivated at all; never starts an activity spontaneously; cannot be induced to take part even on very strong stimulation.

Emotional impairment

	0	1 2	3 4	5 6
Dressing and undressing	Dresses and undresses without help. ☐	Gets help with buttons, zips, etc. ☐ ☐	Requires help from a care-giver to dress and undress but lakes an active part. ☐ ☐	Is completely dependent on a care-giver to be dressed and undressed. ☐ ☐
Meals	Feeds him/herself without help. ☐	Requires supervision and occasional help during meals. ☐ ☐	Constantly requires supervision and help but takes an active part. ☐ ☐	Is completely dependent on a care-giver to be let ☐ ☐
Physical activity	Can walk unimpeded, possibly with a stick. ☐	Requires a walking-aid to be able to walk. ☐ ☐	Requires help from a care-giver to be able to walk. ☐ ☐	Is chairbound or bedridden. ☐ ☐
Spontaneous activity	Normal motor function and spontaneous activity. ☐	Sits still more often than can be considered normal but acts or moves spontaneously on slight stimulation. ☐ ☐	Moves spontaneously only when strongly Stimulated, e.g. when relatives visit him/her. ☐ ☐	No spontaneous activity moves only on direct exhortation, e.g. when told to go the dining-room, to bed, etc. or in response to basic needs (e.g. the need to go to the lavatory). ☐ ☐
Personal hygiene	Manages his/her personal hygiene without help. ☐	Requires some help with shower or bath but manages e.g. washing, combing and teeth-brushing. ☐ ☐	Requires help with all personal hygiene but takes an active part. ☐ ☐	Requires help with all personal hygiene; takes no active part. ☐ ☐
Control of bladder and bowel	Can control bladder and bowel. ☐	Occasional mishaps with micturition but otherwise manages if reminded or promptly helped to the toilet or with a bedpan. ☐ ☐	Mishaps with micturition several times weekly and/or occasional incontinence of faeces. ☐ ☐	Constantly incontinent of urine and/or faeces ☐ ☐

Behavioral and psychological symptoms in dementia (BPSD)

	0	1 2	3 4	5 6
Confusion	Is able to think clearly and has normal contact with people in his/her environment. ☐	Appears bewildered and inquiring. ☐ ☐	Obviously contused; the interview is difficult and time-consuming and the answers often inadequate, but short spells of lucidity occur. ☐ ☐	Completely confused; no meaningful communication can be established ☐ ☐
Irritability	Shows no signs of irritability. ☐	Occasionally shows signs of irritability. especially when asked indiscreet questions. ☐ ☐	Contact not intended to be provocative often produces irritability that he/she cannot always control. ☐ ☐	All contact produces marked irritability that he/she cannot control. ☐ ☐
Anxiety	Shows no excessive anxiety. ☐	Occasionally worried; unnecessarily anxious about various things but can control his/her feelings. ☐ ☐	Constantly excessively anxious; worries about trifles but can be diverted. ☐ ☐	Is so markedly anxious that he/she cannot perform purposeful acts; worries about trifles and can not be diverted. ☐ ☐
Fear-panic	No mental or physical signs of fear or panic. ☐	Occasionally shows signs of vague mental distress but is able to control the condition. ☐ ☐	Continuously shows signs of diffuse mental distress that may develop into panic; the condition is reflected in the body posture; vegetative symptoms. e.g. sweating and palpitation may occur; cannot be diverted. ☐ ☐	Shows signs of marked diffuse mental distress with prolonged panic attacks; feelings of horror and/or extreme agony of death occur; cannot be diverted. ☐ ☐
Depressed mood	Neutral mood level. ☐	Occasionally appears low-spirited and self-reproachful, but spells of brighter mood predominate. ☐ ☐	Markedly depressed, which is evident in speech, facial expression and posture (e.g. feels forsaken by family and friends and complains about pain, fatigue, sleep problems, etc. ☐ ☐	Extremely depressed, which renders him/her incapable of functioning in most situations. ☐ ☐
Restlessness	Shows no signs of restlessness. ☐	Some signs of restlessness, e.g. changes position several times during the conversation, has difficulty in keeping hands and feet still and often fidgets with various objects. ☐ ☐	Appears markedly restless, e.g. cannot sit still and repeatedly makes movements to stand up during the conversation, wrings his/her hands or picks intensely at objects within reach. ☐ ☐	Wanders about almost incessantly; and cannot be induced to sit still even for a short while. ☐ ☐

Results

Keys	Scores on individual items		GBS profile
Intellectual (I), emotional (E) and ADL items: < 1 = normality 1-2 = mild impairment 3-4 = moderate impairment 5-6 = severe impairment Symptoms (S): 0 = absence of the symptom 6 = maximum severity Scores on the intellectual items can be summed up to a *total I-score*. Likewise, scores on the emotional and ADL items can be summed up to a *total E-score* and a *total ADL-score*. However, the scores on the symptoms (S) should not be summed up. No dementia: Total I-score 0-1 Total E-score 0-1 Total ADL score 0-1 Mild dementia: Total I-score 2-24 Total E-score 2-6 Total ADL score 2-12 Moderate dementia: Total I-score 25-49 Total E-score 7-12 Total ADL score 13-24 Severe dementia: Total I-score 50-72 Total E-score 13-18 Total ADL score 25-36 The GBS profile gives a good idea of the extent of dementia in a patient. To use it: fill the relevant circle (score 0-6) for each item. Use a straight line to connect the first filled circle with the second one and then connect the second and third ones and so on within each of the I, E and ADL subscales. The circles relating to the symptom (S) section should not be connected.		Score	0 1 2 3 4 5 6
	Intellectual impairment (I)		
	Orientation to person ……..	…….	O O O O O O O
	Orientation to time ……..	…….	O O O O O O O
	Orientation to space ……	…….	O O O O O O O
	Recent memory ……...	…….	O O O O O O O
	Distant memory ……...	…….	O O O O O O O
	Wakefulness ……	…….	O O O O O O O
	Concentration ……	…….	O O O O O O O
	Ability to increase tempo ……	…….	O O O O O O O
	Absentmindedness ……..	…….	O O O O O O O
	Long-windedness ……	…….	O O O O O O
	Distractibility ……..	…….	O O O O O O O
	Language disturbances ……	…….	O O O O O O O
	Total I-score ……...	…….	
	Emotional impairment (E)	…….	
	Emotional functions ……	…….	O O O O O O O
	Emotional liability ……..	…….	O O O O O O O
	Motivation ……...	…….	O O O O O O O
	Total E-score ……..	…….	
	Impairment of ADL performance (ADL)	…….	
	Dressing and undressing …….	…….	O O O O O O O
	Meals ……..	…….	O O O O O O O
	Physical activity ……..	…….	O O O O O O O
	Spontaneous activity ……...	…….	O O O O O O O
	Personal hygiene …….	…….	O O O O O O O
	Control of bladder and bowel ……..	…….	O O O O O O O
	Total M score ……	…….	
	Behavioural and psychological symptoms in dementia (S)	…….	
	Confusion ……	…….	O O O O O O O
	Irritability ……	…….	O O O O O O O
	Anxiety ……	…….	O O O O O O O
	Fear-panic ……	…….	O O O O O O O
	Depressed mood ……..	…….	O O O O O O O
	Restlessness ……	…….	O O O O O O O

Patient's name: ……

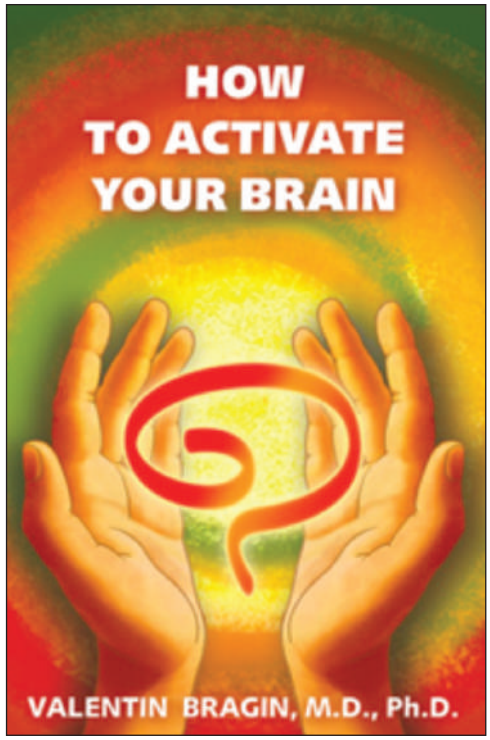
Date of birth: ……..

Date of assessment: ……

Rater's name: ……

How to Activate Your Brain

By Valentin Bragin



How to Activate your Brain is the first in a series of forthcoming books that provide valuable information about how to improve brain performance through fun, simple and easy exercises and techniques, elaborated based on the author's personal research and clinical expertise as psychiatrist, on his experience as the founder and medical director of the Stress Relief and Memory Training Center (SRMTC) in Brooklyn, New York, where more than 2,000 patients have been treated over the past 12 years, as well as on the dedication and the competence of his staff. How to Activate your Brain assume and fulfill its aim of a practical guide for severely disabled older people by the mission it assumed: to educate people about the brain's amazing capacity for self-repair, to help them to utilize their brain power to overcome stress, to improve coordination and increase memory and concentration, and to help people fight depression and anxiety. The techniques described in this valuable and scientifically elaborated brain fitness compendium include breathing and meditation exercises, diet modification, music therapy, simple physical actities and many other techniques, able to improve or restore brain's everyday functions, focus, concentration and memory. The training program outlined in How to Activate Your Brain can be used for many different needs, ranging from fighting stress and emotional problems, to improving concentration and memory. The core of this book lies on the author's strong belief issued from his research and clinical experience that an improvement of brain functioning is real and possible today even for the homebound elderly people. The sensory-motor exercises described in the book addresses the insight that from the birth the development of the nervous system and the brain evolve based on our constant muscle movements and sensory activation. Over time connections between nerve cells become more abundant and fine tuned, having a tremendous impact on the child's growth and maturity, speech, memory, attention and coordination development and training. The same muscles movements and sensory functions are the foundation for the brain activation program for older adults who experience signs of disintegration of the brain functions with aging.

The testimonials of the patients at the Stress Relief and Memory Training Center, as well as several scientific publications, document the beneficial outcomes of the How to Activate Your Brain program on people with different physical and mental disabilities and various levels of memory loss: remarkable improvements of the reaction time (brain speed), attention, memory and coordination. At the beginning of the treatment any mental task requires additional efforts from different areas of the brain, but over time the performance of the mental task improved, became less depended on other areas of the brain, the patient had a positive emotional experience, gain confidence and optimism, and as a significant number of cases prove it, anxiety and depression fad away.

"I have a strong belief that an improvement of brain functioning is real and possible today even for the homebound elderly people. Activation and improvement of the brain's functions are possible through sensory-motor exercises", says Valentin Bragin while realizing his first practical approach of this concept in his book.

Supported Human Autonomy for Recovery and Enhancement of cognitive and motor abilities using information technologies

SHARE-it

A STREP project number FP6-045088, co-funded in the EU's FP6, in the IST (Information Society Technologies) Thematic Priority, started on January 1st 2007, fulfilled by 8 partners from 4 European countries until 2009.

THE PROJECT

SHARE-it goal is to develop a scalable, adaptive system of add-ons to sensor and assistive technology so that they can be modularly integrated into an intelligent home environment to enhance the individual's autonomy.

The system will be designed to inform and assist the user and his/her caregivers through monitoring and mobility help. It is planned to contribute to the development of the next generation of assistive devices for older and/or disabled, cognitively including, people, so that they can be self-dependent as long as possible. It focuses on add-ons regarding the compatibility with existing technologies and the achievement of an easier integration into existing systems. Adaptive and as easy as possible to use are envisaged. Scalability is meant to include or remove devices from the system in a simple, intuitive way.

SHARE-it addresses important issues in sensor networks, assisted mobility, knowledge engineering and Ambient Intelligence, making significant contributions to fundamental, long-term research in the following areas:

- C1: Sensor-based environment perception, knowledge acquisition / representation, high-level reasoning and goal seeking behaviors in a real world (preferred environment).
- C2: Verifying software adaptation to human with special needs: both at design and run-time - as operating conditions and governing norms change –
- to establish (e.g.) safety, regulatory and security requirements.
- C3: Incorporating shared autonomy: ensuring individual software components and groups of software components can be designed to operate
- in a given intelligent ambiance and adapt to possible changes both in the needs of the user or in the environment.

The objectives of SHARE-it are:

- O1: To explore the benefits of the concept of situated intelligence to build elements (add-ons) that will enhance the autonomy of the target user group in their daily life in their preferred environment.
- O2: To investigate and implement innovative forms of shared autonomy.
- O3: To build appropriate add-ons to standardized technologies to provide ubiquitous sensing, computation and assistance
- O4: To build adaptive interfaces for the target group.
- O5: To target the various human-delivered assistance and caretaking services as effectively as possible.

The overall project work is intended to bring together existing work from a number of areas and make a concerted effort to develop a sound paradigm for future development of mission critical systems for individuals suffering disabilities involving automated components: creating important reference points in the field, aiming to catalyze debate and opening future research directions.

Project Coordinator: *Universitat Politecnica de Catalunya*, Professor Ulises Cortés

ANA ASLAN INTERNATIONAL ACADEMY OF ANTI-AGING

ANA ASLAN INTERNATIONAL FOUNDATION

CONTRIBUTION AS MEDICAL PARTNER, DURING THE 1ST YEAR – 2007

- **Share of knowledge and expertise regarding :**
- **"State of the art" in intelligent assistive devices**
- **Stipulations contained in the International Plan on Aging and other UNO and WHO documents**
- **Problems concerning the compliance of the frail elderly and cognitively impaired persons to the usage of such assistive devices**
- **Presentation of the scales suitable to define the Target Population** (SHARE-it's Workshop *"Aging of the population, chronic conditions, disability and their assessment"* from Rome)
- Meanings about the psycho-physical functions of the patients with cognitive impairments (e.g. moderate-severe to severe Alzheimer's Disease and other related dementias,)
- Meanings about the psycho-physical functions of the patients with cognitive impairments
- Information regarding patophysiological changes due to cognitive impairment provided for "*Definition of the user and activity, tasks and ADLs*"
- Provision of a set of up-to-date assessment and evaluation scales for "*Protocols of user assessment*" design: Clinical / Physical Exam; GSE, MMSE, MNA, Tinetti Static & Tinetti Dynamic Scales, Yesavage Geriatric Depression Scale, GBS, IADL, SIB for the demented patients with MMSE <10, RUD, etc.
- Dissemination of SHARE-it promotion materials in Romania as well as on our ANA ASLAN INTERNATIONAL website, following the link www.brainaging.ro/pdf/RD1.pdf
- Translation in Romanian of SHARE-it's *Fact Sheet* and *Press Release* and their publication in Brain Aging International Journal (Editor in chief: LUIZA SPIRU, MD, PhD), Vol. 1, 12.11.2007
- Presentation of SHARE-it's the public area information, as well as of ANA ASLAN International Academy Of Anti-Aging's contribution, to the Romanian Ministry of Health.

SHARE-it Project's promotion during the International Training Workshops on Memory Impairment" , Bucharest, Infoturism, September 22, 2007 http://www.brainaging.ro/Training.htm and Bucharest, Parliament Palace, May 5th, 2007, and during *Press Cconferences* with *TV, Rompress Agency, and news-papers: "Jurnalul National", "Bussiness standard", "Gandul", "Ziua", "Cotidianul", "Newsin"*, at : *"Carol Davila" University of Medicine and Pharmacy*, Bucharest, Romania, October 06, 2007, Bucharest *Infoturism*, September 22, 2007 and Bucharest *Parliament Palace*, May 5th, 2007.

Romanian Team Coordinator: Luiza Spiru, MD, PhD

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Co-morbidities as a Hot Topic in Predementia Conditions. A Romanian Neuroepidemiological Study.

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As the population ages, Alzheimer's disease and depression are becoming increasingly common concerns for primary care physicians. The comorbidity of Alzheimer's disease presents a complex diagnostic and management challenge. The causes of death for persons with AD may vary, depending on the stage of cognitive impairment in the patient at the time of death. In a study of patients with mild to moderate cognitive impairment, the causes of death (stroke, cerebral hemorrhage, neoplasm, and cardiovascular disease) differed from that found in patients who died later in the progression of AD, in whom bronchopneumonia was noted the most common death cause. Comorbid conditions are vulnerable to under diagnosis and misdiagnosis, mainly due to patients' inability to communicate .

The study was fulfilled on 60 MCI (Mild Cognitive Impairment) subjects (75% aged 60-75 years, 77% women and 23% men, 37% university graduated and 90% from urban area), at "Elias" University Emergency Hospital from Bucharest – Romania, as the first Romanian study of this kind based on internationally recognized diagnostic criteria and follow-up methods. It attempted MCI to AD conversion challenges: clinical criteria, risk factors (RF), co-morbidity prevalence, validation of the international pattern of RF distribution in MCI patients, having as main objectives: **development of clinical criteria for predementia Alzheimer's disease (AD); assessment the risk factors and co-morbidity prevalence in MCI patients; validation of the international pattern of RF distribution in MCI patients.**

The study represents the Romanian contribution to the "Development Of Screening Guidelines And Clinical Criteria For Predementia Alzheimer's Disease – DESCRIPA" Study, developed by the European Alzheimer Disease Consortium - EADC.

The obtained results revealed an MCI to AD conversion rate of 23,3% at the first year follow-up. In the next two years, another 6.6% patients developed AD. Hypertension, angina pectoris and dyslipidemia were the most prevalent comorbidities, followed by diabetes type II, osteoporosis senile, transient ischemic attack, hypothyroidism and carotid artery stenosis. The vascular factors hypertension and dyslipidemia exhibit a shape almost similar to that documented in AD patients, being a possible indication for their importance as main risk factors for AD development in MCI patients in the Romanian study. There is a sex-dependent co-morbidity distribution. At baseline all comorbidities prevailed in women (except transient ischemic attack). During the 3 years of follow up the number of women exhibiting hypertension, dyslipidemia, angina pectoris, transient ischemic attack, and diabetes type II was significantly decreased , while the number of those afflicted by osteoporosis and hypothyroidism increased. The lack of significant changes in the co-morbidities' distribution in men, as well as the slight numeric increase of male patients exhibiting hypertension and transient ischemic attack need further clarification. The incidence of the depressive mood disorders was also found to depend on sex. It significantly prevailed in women (13%) by comparison with men (3%) at baseline, exhibiting a rise in the former from 13% to 20% during the follow up, while no percent modification was registered in men. As being common in preclinical AD, depression needs to be differentiated from that of the subjects with depression-related cognitive impairment. The total amount of patients afflicted by depression in the Romanian study (23%) could suggest that the subjects with this condition should not be excluded from the cohorts selected for preclinical Alzheimer's disease studies.

Brain Aging International Journal starts its "At a Glance" column, dedicated to the signaling of the latest news in the field of normal and pathological brain aging

International Conference on Alzheimer's Disease – ICAD 2008

Highlights of Research Findings

Does one protein mechanism underlie the development of plaques and tangles? Which risk factors most accurately predict the development of dementia? How does the communication style of caregivers affect the quality of life of individuals with Alzheimer's disease (AD)? The Alzheimer's Association 2008 International Conference on Alzheimer's Disease (ICAD), held July 26–31 in Chicago, sought to answer these and other questions in diverse areas ranging from drug trials to genetics, neuroimaging, diagnosis and social and behavioral issues in AD and other forms of dementia. Historically the world's largest gathering of AD and dementia researchers, ICAD 2008 broke previous attendance records, drawing more than 5,400 attendees to more than 2,000 plenary, symposium, oral and poster presentations. The conference attracted media attention both in the United States and abroad, with coverage by outlets including ABC, the BBC, CBS, CNN, NBC, the Associated Press, Reuters, The Wall Street Journal
and USA Today. Opening the conference, Alzheimer's Association President and CEO Harry Johns thanked the attendees for the work they do every day to unlock the mysteries of dementia and shared some of the Association's strategic goals, including "raising Alzheimer's from a disease to a cause that is embraced worldwide," increasing financial support to researchers, and enhancing advocacy efforts to heighten awareness of the epidemic of AD. The disease and other dementias cost the United States more than $148 billion annually in Medicaid and Medicare services and in indirect costs to businesses that employ AD and dementia caregivers. According to one study, providing care for the estimated 29.3 million people worldwide with AD cost $315 billion in 2005. To accelerate the pace of research and the sharing of research advances, Johns announced that the usually biannual ICAD will be held annually. The 2009 meeting will take place July 11–16 in Vienna, Austria.

Disease mechanisms and therapeutic strategies

Researchers' and clinicians' keen interest in the development of drugs that will slow or stop the progression of AD was especially evident on the
second day of the event, when a symposium on disease-modifying drugs drew a full house. The symposium featured six speakers who reflected the global focus on disease modification. The speakers and their topics were Bruno Vellas, M.D., Ph.D., of Purpan-Casselardit Hospital in Toulouse, France (Recommendations and Outcomes of Disease-Modifying Drugs); Eric Siemers, M.D., of Ely Lilly and Company in Indianapolis, Indiana, United States (Disease Modification: Will We Know It When We See it?); Yasuo Ihara, M.D., University of Tokyo, Japan (Why a Beta-Amyloid Vaccine?); Colin Masters, M.D., Ph.D., of the Mental Health Research Institute, University of Melbourne, Parkville, Australia (Rational Therapeutic Strategies for Modifying AD: Beta-Amyloid Oligomers as Validated Targets); Roger Nitsch, M.D., of the University of Zurich, Switzerland (Beta-Amyloid Immunotherapy in AD); and Bengt Winblad, M.D., Ph.D., of the Karolinska Institutet, Stockholm, Sweden (Safety, Tolerability and Immunogenicity of the Beta-Amyloid Immunotherapeutic Vaccine CAD106 in a First-in-Man Study in AD Patients). ICAD 2008 gave attendees insight into the broad range of drugs in clinical trials. They incorporate an array of approaches to impact biological processes associated with AD. "The overarching message is how robust the pipeline is," said speaker Sam Gandy, M.D., Ph.D., of the Mount Sinai School of Medicine in New York, New York, United States. "Research is moving on all fronts and in unexpected directions."

• A six-month open-label extension trial of Dimebon produced results similar to those in the preceding 12-month clinical trial. Patients with mild-to-moderate AD who had earlier received the drug for 12 months had

preservation of function close to their starting baseline on key signs and symptoms of AD. Patients originally on placebo who received Dimebon in the extension study showed stabilization across all key measures studied. Originally developed in Russia as an antihistamine, Dimebon improves the function of mitochondria, the central energy source of cells. Recruitment for a Phase III study has begun.

• Treatment with intravenous immunoglobulin (IVIg) over nine months resulted in statistically significant improvements on both cognitive and global clinical measures in a Phase II trial of individuals with mild-to-moderate AD. On the market for more than 25 years as a treatment for autoimmune diseases, IVIg contains antibodies that bind to the beta-amyloid aggregates thought to be central to AD. A Phase III clinical trial is under way.

• A 24-week, Phase II trial of methylthioninium chloride (MTC) followed by a 60-week extension trial found that at 24 weeks MTC produced a significant improvement relative to placebo. The compound stabilized the progression of AD over 50 weeks in both mild and moderate AD. MTC, which dates from the 1930s, inhibits the aggregation of tau, the protein that forms the neurofibrillary tangles of AD. Among its earlier uses, it was used as an antibiotic. A Phase III trial is planned. Paul Aisen, M.D., of the University of California, San Diego, United States, discussed the significant challenges facing those who conduct clinical trials. Among the challenges is the slow decline of placebo groups in clinical trials. For a drug to show that it stops or slows the progression of AD, the group that receives a placebo must far worse. This requires longer trials lasting at least 18 months, because that's how long the placebo group needs to show a decline. Phase III clinical trials, which can cost $200–$300 million, still carry a high risk of failure because of the potential of larger sample sizes and longer trial durations to produce results different from smaller, shorter Phase II trials. Strategies to employ in Phase II trials to decrease the risk of Phase III trials include aiming for "hints of clinical efficacy" and showing proof of the drug's mechanism of action through biomarker studies. The physical changes to the brain in AD begin years before clinical symptoms such as memory loss develop. Disease-modifying drugs will likely be most effective before individuals develop clinical symptoms. A significant task before the research community is showing that impacting putative biomarkers of AD, such as levels of beta-amyloid in cerebrospinal fluid (CSF) and patterns of brain loss seen on imaging tests such as magnetic resonance imaging (MRI), results in cognitive benefit in the clinical setting. Doing so could elevate biomarkers to the status of surrogates for AD in the eyes of regulatory agencies that establish the requirements for clinical trials. This would enable researchers to set biomarker changes as evidence of a clinical trial's success. "There is a movement to identify the disease earlier and earlier, and we need presymptomatic biomarkers to do this," said speaker Ronald Petersen, M.D., of the Mayo Clinic, Rochester, Minnesota, United States. "A consistent theme has emerged of early detection for early intervention."

Biomarkers

Biomarkers have been an area of intense focus by researchers. Identifying biomarkers could lead to the development of simple tests such as blood tests that would be easy to use in the clinical setting and more readily accepted by the public than more complicated tests. Neuroimaging, which is able to detect changes as small as one millimeter in the structure of the brain, can show both the degree of brain loss at any given time as well as the rate of loss of brain volume over time. Some presenters believe rate of brain loss may be more important than brain volume alone in the early detection of AD and other dementias.

• Researchers reported that levels of CD-69, a protein involved in white blood cell growth and production, were more than 80 percent accurate in distinguishing those with AD from those who were cognitively normal and more than 90 percent accurate in distinguishing individuals with AD from individuals with Parkinson's dementia. Healthy brain cells do not undergo the process of division and replication (the cell cycle) that is common to other cells in the body. In AD, however, brain cells may prepare to reenter this cell cycle, which may increase the likelihood of cell death. The same cell cycle defect is found in the white blood cells of people with AD.

• According to researchers, computer analysis of MRI scans can accurately capture the severity of AD-related neurofibrillary tangles. The analysis is used to establish a score on the new Structural Abnormality Index (STAND). A STAND score is assigned by comparing the degree of atrophy in an individual's brain with atrophy patterns of 160 individuals with AD and 160 cognitively normal persons. Researchers recorded STAND scores for 101 individuals before death and compared them with scores from postmortem BRAAK staging, the "gold standard" for assessing tangle severity. The STAND scores were 90 percent accurate in distinguishing MRI scans of individuals with AD from cognitively normal individuals.

Risk factors and prevention

Beginning in the mid-1990s, large epidemiologic studies were undertaken to identify factors that contribute to brain health as well as to cognitive decline. These studies had consistent findings, showing that factors such as physical and mental inactivity were associated with higher risk of

cognitive decline. However, these studies were conducted with individuals in late life and lasted for only a few years. More recent studies provide data from individuals beginning at mid-life and ending in late-life.

• Using risk factor information from a pooled European database of more than 16,000 nondemented individuals over age 55 and conducting follow-up studies up to 15 years later, researchers determined the risk factors with greatest accuracy of predicting dementia. In order, the most predictive variables were impairment in executive function (planning), memory problems as measured on tests, subjective memory or cognitive complaints, apolipoprotein e-4 genotype, use of psychotropic medication, severe head trauma, diabetes, stroke and language difficulties.

• A study of 422 healthy elderly persons over age 60 showed that those with metabolic syndrome had an almost 35 percent higher level of cognitive compromise than those without metabolic syndrome, a group of heart disease risk factors that includes abdominal obesity, high blood pressure, high triglycerides, high blood sugar and low HDL cholesterol. Researchers used a battery of scales to assess cognition, depression, planning abilities and activities of daily living. Individuals with metabolic syndrome had significantly lower scores on all neurofunctional tests, reinforcing the importance of good physical health in reducing one's risk for cognitive decline. Communication style and quality of life The quality of life of individuals with AD and other dementias is affected by numerous factors. Some factors are well known, such as the importance of the individual continuing to engage in enjoyable activities and creating a safe physical environment for the individual. Less well known is the effect of caregiver communication style on quality of life. However, researchers have discovered that the impact is significant.

• "Elderspeak," defined as overly caring, controlling and infantilizing communication, by caregivers increases resistance to care by nursing home residents with dementia, said researchers. Individuals with dementia were more likely to cooperate with care activities such as bathing and dressing if normal adult communication was used. The probability of resistance to care was .55 with elderspeak and .26 with normal communication.

• As AD progresses, individuals have increasing difficulties with communication. These difficulties are related to cognitive changes such as impaired word finding, shortened attention span and impaired memory. A study from the University of California at Los Angeles found that healthy family members' responses to unanticipated comments from individuals with AD followed predictable patterns. When a response disrupted the flow of conversation, healthy family members often continued to speak as if the person with AD had not spoken or tended to pause, indicating they had heard the comment, but did not respond verbally. Such responses frame the individual with AD as a nonparticipant in the conversation. The results of the study will be used to develop training programs to facilitate conversation among all family members.

For more research news from ICAD 2008, visit http://www.alz.org/media_12490.asp. To learn more about obtaining CD-ROMs of ICAD presentations, please visit the main ICAD page at www.alz.org/icad.

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