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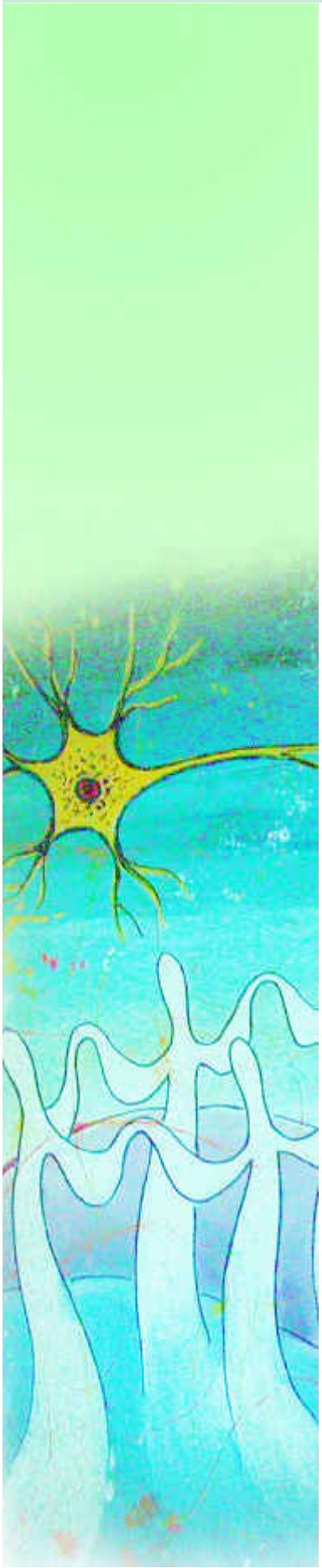
ISSN 1582-8352 (printed)
ISSN 1582-8360 (on-line)

Brain Aging International Journal®
Ana Aslan International Academy of Aging

The accepted abbreviation for **Brain Aging International Journal** for bibliographic citation is **Brain Aging**

Scientific board



Remarks on Aging and Senescence

The process of aging has been defined as an ordinary, progressive change in the metabolism of an individual organism with the passage of time, the culmination of which is life or death. This concept would suggest that in human being aging begins in the moment of conception and progresses relentlessly through intrauterine life, childhood, adolescence, adulthood and advanced age. In human beings, however, it is generally recognized that certain manifestations of biologic maturity are achieved at different ages and that these do not necessarily occur simultaneously. For example, growth in stature usually ceases at 17 to 20 years of age, whereas sexual maturity may be achieved at an earlier age. The rate of learning may reach its maximum level at an early age, whereas judgement, perception, and evaluation may not be fully developed until later in life. Thus, although a relationship between time and the aging process may appear in single celled organisms such as bacteria or amoebas, a similar relationship does not apply in multicellular vertebrates such as human beings. Furthermore, it must be recognized that the processes of growth and aging are actually opposite to one another. Growth contributes in a positive way towards the organism achievement of maximum size and functional efficiency, whereas aging reflects the influence of adverse changes that interfere with the ability of the organism to function efficiently in its environment. As a result of such considerations, **Lansing has suggested that aging or senescence should be defined as "a process of unfavourable progressive change, usually correlated with the passage of time, becoming apparent after maturity and terminating invariably in the death of the individual".**

Studies of culture techniques of human tissues have served to demonstrate that some tissues, such as fibroblasts, do not have the capacity to multiply and replicate indefinitely, even under ideal conditions. Human fibroblasts survive fifty sub-cultivation and then die. It has been suggested that with each successive generations in DNA and/or RNA may occur something which eventually leads to death of the cell line. It must be recognized that in human beings certain cell lines continuously replace themselves. The life span of the erythrocytes occurs. In contrast, in other organ systems such as skeletal and cardiac muscles, the central nervous system, the kidney and the pancreas, the maximum number of functional units is achieved at maturity, and thereafter the passage of time is associated with a progressive reduction in the number of functional units without replacement.

As a result of numerous observations in Paramecium and Drosophilae, as well as in other genera, data have accumulated to suggest that aging, and therefore senescence, reflects a systematic programming of somatic mutations. The determinants or the „Input" for such a programme are introduced through the genetic material each individual receives from his ancestors. In addition, the „programme" may be influenced by environmental factors such as trauma inflicted by radiation, temperature variation, infection or dietary factors.

Aging processes, therefore, have been classified as primary and secondary processes. Primary aging refers to those changes that relate to diminishing functional capacity associated with the passage of time. Secondary aging represents the diminution of functional capacity that stems from disease or trauma. Obviously, in a given individual a clear identification of primary and secondary aging may be very difficult and, indeed, the two may be closely interrelated. Since the rate of primary aging may be modified by environment and diseases, secondary aging per se may hasten the process of primary aging.

According to our present state of knowledge if all causes of death related to trauma or to disease processes could be eliminated, death would result as the simple consequence of aging or senescence. This would suggest that information regarding the aging process could be obtained through histological and biochemical studies of tissues from individuals and various ages. A vast amount of information of this kind has accumulated over the years. Unfortunately, in many instances such data have been obtained from individuals suffering from a variety of diseases, or the analysis have been conducted on tissues obtained at the time of autopsy and therefore the

values obtained had been modified both by agonal and post-mortem changes. The most extensive data collected on the aging process concern the central nervous system. These data indicates that the human brain achieves its maximum weight by the age of 30 years. Therefore, a gradual loss of total brain weight occurs with the passage of time. Based upon such findings the age of 70 has been chosen as the time of onset of senescence.

Although, total brain weight continues to decrease after this time, the water contain of the brain increases more rapidly while the content of solids and total lipids decreases strikingly. The decreases in tissue solids include decreases in total nitrogen and phosphorus content. The content of DNA however increases during this period and this change is linked to the presence in the brain of an increase of glial cells. Paralleling with the increase with the DNA contain of the brain is the increase of the sulphur which is associated with an unidentified lipid substance and this inturn may be related to the occurrence of the yellow plaques that appear with increased frequency in the aging persons' brain. Studies of cerebral blood flow and cerebral oxygen consumption in persons without significant cerebrovascular diseases indicate that the changes which occurred in senescence are probably related most closely to the reduction of the total number of neurons. As previously indicated, the overall morphology of the brain changes with aging includes a decrease in total brain weight, evidence of atrophy of the cortex and dilatation of the cerebral ventricles.

Histological, the total number of neurons decreases, although this decrease does not correlate with age or with apparent cerebral function.

Through the application of such techniques as electron microscopy and other methods of approaching subcellular structure, it is probable that more precise data regarding the mechanism of aging may be achieved. Estimates of lean body mass by isotope dilution techniques utilising tritium or deuterium as well as total body counting techniques, have served to demonstrate that beyond maturity there is a gradual but progressive decrease in the lean body mass and, thus, in the muscle mass of the body. Although such diminutions in muscle mass are undoubtedly accelerated by systemic diseases, they are also observed to occur in individuals who have not experienced serious diseases of any kind.

Similarly although adequate data are lacking, total renal function deteriorates with advancing age. Under normal circumstances, such diminished renal function is not recognised. When renal functional capacity is challenged in the older patient by such as shock, massive gastrointestinal haemorrhage, or prostate obstruction, however, a rapid rise in blood area nitrogen may occur with the concomitant manifestations of uraemia.

The aging of the individual who has inherited diabetes is characterised by a progression from the stage of clinical diabetes. Thus, with advancing age, there is a progressive loss of islet tissue and a gradual alteration in the ability of the individual to metabolise glucose.

Calendar age versus biologic age

For purposes of the physician, age may be measured in terms of calendaristic years. This often has little significance, however, because the functional, biological, or medical age of an individual may be quite different from actual, or calendar age. Over the centuries mankind has witnessed a paradox with reference to aging. On the one hand, many of the leaders of our current civilization, as in past civilizations, fall into the category of aged citizens in terms of the calendar, but nevertheless they have become members of an "elite corps" of great leaders. Among recent leaders who have gained such distinctions can be mentioned Winston Churchill, Douglas MacArthur, Konrad Adanauer, Dwight D. Eisenhover, Igor Stravinski and Pablo Casals. At the same time, it is possible to visit the wards during any day of the week and observe a vast group of individuals of the same age as these leaders (and often younger) who are literally vegetating and who are on the hopeless downhill road to the dependency and eventual death. What is the difference between these two groups of individuals, world leaders and end-of-the-roaders, at the ages 65, 70 and 75 years or over? Although objective evidence is lacking, it is obvious that time and the exigencies of living affect men in different ways. One possible explanation lies in the fact that each of these leaders has been in a position to be protected from many of the stresses of living.

Information has accumulated to demonstrate that overall life expectancy is basically determined by genetic factors and that this is undoubtedly controlled by multiple genes. Inheritance, in turn, is influenced significantly by environment. If any of the world leaders mentioned had been afflicted by a serious attack of the many infection diseases prevalent during the time of his youth, he would not have survived to maturity and the genetic influences resulting in longevity could not have played an important role in determining longevity and environmental factors, including infectious diseases, trauma, physical and chemical factors, and a host of other agents may serve to deny the influences of heredity. Each decade has witnessed great progress in medical science that have served to extend life expectancy. At the same time, other factors are involved that have served to partially deny this progress. Many of us live in what has been an "affluent society" and neoplastic diseases are commonest in an aging population.

Aging and stress

On a statistical basis, acute diseases (both medical and surgical) are no more common in aged than in young individuals. However, the response of aged persons to stress as represented by acute distress is frequently quite different from that seen in younger individuals. An aged person, for example, may experience only light abdominal discomfort with relatively little fever or leukocytosis in the presence of

an attack of acute appendicitis. Why the mechanism diminishes in elderly persons is not completely understood at present, although such responses undoubtedly reflect the effects of involutionary changes in various organ systems such as the bone marrow and the adrenal cortex, which show characteristic responses to stress in younger persons. Diminished responses may be disadvantageous to the older persons in the presence of acute infections or some acute abdominal condition requiring surgical treatment in that the physician may fail to make the correct diagnosis promptly. On the other hand, these same mechanisms make the aged individual more tolerant to surgical procedures and, as a result, such persons may exhibit minimal reactions to major surgery. On the contrary, disability resulting from some acute diseases is often extended in elderly individuals, leading to longer periods of disablement. In other words, diseases that produce short periods of disability in younger individuals may cause extended disability in aged persons.

Aging and chronic diseases

Chronic diseases tend to be far more common in the aging population than in younger age groups. Such diseases include diabetes mellitus, atherosclerotic heart disease, strokes, cancer, various forms of arthritis and hypertensive disease, together with their complications. In terms of the aging process, these may be regarded as secondary factors, which influence upon the normal course of aging.

Parameter of degenerative change

It has been suggested that the calendar alone does not uniformly provide an adequate measuring stick to evaluate the deterioration that accompanies aging. The definition of biologic aging indicates that certain metabolic changes occur within cells which ultimately lead to the death of such cells. This would suggest that biochemical analyses of such metabolic processes might provide a means of measuring the degree of aging. Unfortunately, with few exceptions, such parameters for the estimation of aging are not currently available. As a matter of fact, the values for ordinary laboratory determinations in healthy aged individuals are identical to those observed in younger persons. Another striking aspect of aging is that structural changes often correlate very poorly with biologic or functional standpoint, but the lens of the eye may reveal only limited structural alterations. In contrast, as previously mentioned, marked losses of neuronal tissues may not be accompanied by any easily detectable loss of cerebral function.

On occasion it has been incorrectly concluded that atherosclerosis is a major causative factor of aging. It should be recognised that atherosclerosis is a disease, which may contribute to the aging process, and which occurs frequently in elderly persons, but that it is not, of itself, a primary cause of aging.

On physical examination, a variety of stigmas have been classically associated with the aging process. These include such findings as graying of the hair, baldness, the presence of arcus senilis, the appearance of such skin lesions as senile keratoses, the loss of elasticity of the skin, and the appearance of wrinkles. Unfortunately, some of these, including graying of the hair, baldness, and the appearance of arcus senilis, often correlate poorly with the actual age of the individual. For example, gray hair and baldness may being to appear in the elderly teens, and arcus senilis can be noted in individuals in their 30's and 40's.

The most striking and most disruptive of the changes that accompany aging are the alterations in the central nervous system. As indicated previously, it has been documented that, with advancing age, the brain decreases in size in a progressive fashion and that this is reflected in a decrease of total number of neurons. With such progressive and irreversible loss of brain tissue, it is obvious that intellectual defects should be anticipated. In many elderly subjects, however, such defects cannot be detected by ordinary means, and it is assumed that the remaining cerebral structures serve to compensate for the segments lost. Nevertheless, there are measurable changes that occur with aging, including a delay in neuromuscular response period, a decline in intelligence (which is more evident in persons who originally had lower level of intelligence than in those who had higher levels), a decrease in learning ability, and a loss of memory, particularly for complex materials. Changes in perceptive ability, including a decline in visual and auditory acuity, are also characteristically observed in aging persons. With these measurable decreases in the functional capacity of the central nervous system which occur with advanced forms, the changes in the central nervous system during senescence can be easily recognised.

They are characterised by the appearance of personality changes such as mental apathy, marked irritability, garrulousness, loss of concern for physical cleanliness, and often a complete psychological withdrawal from the environment. Although some of the alterations that accompany aging can be corrected (for example, by dyeing the hair, wearing a toupee, wearing corrective lenses, and taking insulin or vitamin B12), the basic process of aging continues relentlessly, and, once intellectual function has been lost, there is at present no means of replacing it.

At the turn of the 20th century, Sir William Osler regarded pneumonia as "the old man's friend". Until our understanding of the aging process is significantly advanced, we should perhaps regard cancer, strokes, and brain aging in the same category.

Luiza Spiru *MD, PhD*
Editor

"Ana Aslan"
International Academy of Aging

Welcome to the Academy

My colleagues and I are delighted to welcome you to membership in „Ana Aslan“ International Academy of Aging. You will join people from more than 50 countries all over the world, who are members in this organization, committed to medical science, education, culture and art.

The Academy proves itself to be committed to medicine, especially through creating excellent educational and clinical centers for elderly, in accordance with the agreements of World Health Organization, European Union, International and European Consortium in the field of Aging.

You will join a society of scientists, which was founded in 2000. We will invite you to all the conferences, symposia, carried out yearly, which cover all medical, social and educational disciplines.

„Brain Aging“ International Journal issues are available free of charge for years 2001-2002. You may also visit our web site or our on-line journal web site.

We are looking forward to your joining our Academy!

Bengt Winblad MD, PhD
Khalid Iqbal PhD
Luiza Spiru MD, PhD

BRAIN AGING
INTERNATIONAL JOURNAL

„Ana Aslan“ Academy of Aging has the honour to introduce to you probably the most interesting new millennium International Journal, „Brain Aging“.

Ana Aslan was the first one, all over the world, who defined, in 1953, Geriatrics and Gerontology as the most futuristic former millennium clinical specialization.

This great lady, who had the vision in the early 50-ties to define „gerontoprophilaxy“ as the advanced weapon to fight against aging, said that „human life is artificially shorted, but anyone can discover the secrets of longevity“!!

We are pleased to publish under the auspices of „Ana Aslan“ Academy of Aging, the latest news from all over the world, in this so incandescent scientific field, like „brain aging“ is.

This is a journal, which attempts to explain, in a clear and scientific language, how this knowledge may one-day possibly be used to control and overcome brain aging!

We have the mission to encourage also young researchers in publishing, in this field of activity, to grant the most talented of them, every year!

Hopefully, BRAIN AGING will be the host of publishing revolutionary papers, warm scientific information, and Brain Aging scientific timetable.

Read a new Journal, HAVE A NEW FRIEND!!!

Luiza Spiru MD, PhD

"Ana Aslan"
International Academy of Aging

Organisation's profile

Under the care of *„Ana Aslan International" Foundation* the following Academy, called *„Ana Aslan" International Academy of Aging*, is set up, organized and works under the form of a high scientific and cultural instance calling together personalities of outstanding value in the fields of science, technics, education, culture and art, personalities acknowledged on national and/or international level.
The purpose of *„Ana Aslan" International Academy of Aging* is to get established and work as a high scientific and cultural instance, an excellence centre to set up, organize and support projects and programmes for people of age in the scientific, cultural, educational and communication fields.
„Ana Aslan" International Academy of Aging will be able to set up and organize branches in Romania, Sweden, Greece, Israel, Thailand, Germany, as well as in other countries settled by the authenticated decision of the leading Counsel of *„Ana Aslan International" Foundation*.
„Ana Aslan" International Academy of Aging will promote the co-operation between academies, universities, similar institutions and structures in other countries by maintaining direct relations with them, as well as with international organizations of people of age.
The norms regarding the organization and working of the Academy, including the Speciality Committees and their competences will be settled by the Rules of Organization and Working, approved by the Leading Counsel of *„Ana Aslan International" Foundation*, in the quality of *Scientific Counsel* within the Academy, having the same structure as the Leading Counsel of the Foundation.
The Scientific Counsel of *„Ana Aslan" International Academy of Aging* settles the general strategies and the programmes of this scientific and cultural instance, the conditions for appointing the members, as well as the forms in which the Academy will contribute to the achievement of purpose and objectives of *„Ana Aslan International" Foundation*.

Academy Board

Board of Governors:

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Honorary President (deceased):	Prof. Dr. Ana Aslan
Secretary:	Gunilla Johansson

"Ana Aslan"
International Academy of Aging

Who we are

The objects of the Foundation „Ana Aslan International“ are:

a) initiation and financing of programs concerning the development of the Romanian Geriatrics experience and tradition in the therapy of prevention of aging, in accordance with the present medical norms, in Romania as well as abroad;

b) initiation and financing of programs / scientific research projects concerning the reactions of the ASLAN therapy and of the ASLAN geriatric principles, in the frame of the present medicine and their integration in the international geronto-prophylaxis therapy;

c) supporting and supplying additional financial resources for operating the nurseries for elderly and the social institutions by offering sponsorships and donations in cash or in kind;

d) establishing, organizing and financing, according to the law, of prophylaxis geriatric centers, day-care geriatric assistance, medical offices, individual, grouped or associated, within the medical centers in the city of Bucharest and in the territory, where will be employed: specialist geriatric medicine doctors, psychologists, social assistants, physio-kinetotherapists and other personnel;

e) initiation, support and financing of programs for rehabilitation of elderly, by common action of geriatric assistance services and of the balneo-physiotherapy and rehabilitation;

f) initiation, support and financing of programs aiming at reinvigorating of the Romanian geriatrics, of the traditions of the ASLAN therapy and of ASLAN products, in relation with present science;

g) organizing and operating, according to the law, of medical assistance activities and medical assistance and care for elderly people with total or partial autonomy loss, in medical, socio-medical and psycho-emotional fields.

h) initiation, support and financing of projects and programs for elderly persons, concerning:

- social reintegration, juridical and administrative counselling of elderly persons in need;
- their participation in economic, social and cultural activities;
- support for the payment of current services and obligations, home care, household assistance for elderly persons;
- medical visits and care at home, treatment with the prescribed medicine, granting of sanitary materials, medicines and medical devices;
- hiring of personnel for sickroom services for elderly persons.

i) organization and operation, according to the law, of medical assistance services for persons with no income or with income under the limit of the minimum salary in the country;

j) support and financing of projects and programs for people with no income or having an income under the limit of minimum salary in the country, concerning:

- prevention of social marginalization and assistance the social reintegration of these categories of people;
- their participation in economic, social and cultural activities;
- educational programs concerning qualification, re-qualification and professional reintegration;

k) editing, support and promotion of the publication International Journal of Ageing, under the aegis of the Foundation, with an editorial board established by common agreement of the founders members and the international geriatrics community;

l) acquisition of equipment, books, publications and outfit for medical universities and for scientific research in Geriatrics;

m) establishing a club for the meetings of the founders members, honorary members and their guests;

n) support and development, according to the law, of educational structures able to contribute to the facilitation and optimization of social and professional integration of people with no income, of disadvantaged, marginalized young persons, who leave the placement centers or having special needs;

o) granting of sponsorships and donations in cash or in kind for the families with reduced income, sole parents or disorganized families, aiming at facilitating their access to lodging, to help covering the expenses for preparation of children at the starting of the school year and for their access to education and formation services.

p) preparation and promotion of programs for equal opportunity employment for women, and for facilitating the access to hiring, professional formation, scientific, social and cultural recognition;

q) providing the material resources for organizing scientific, cultural and sports activities to the benefit of students, professors, medical doctors and other persons with activities in the fields of interest for the Foundation;

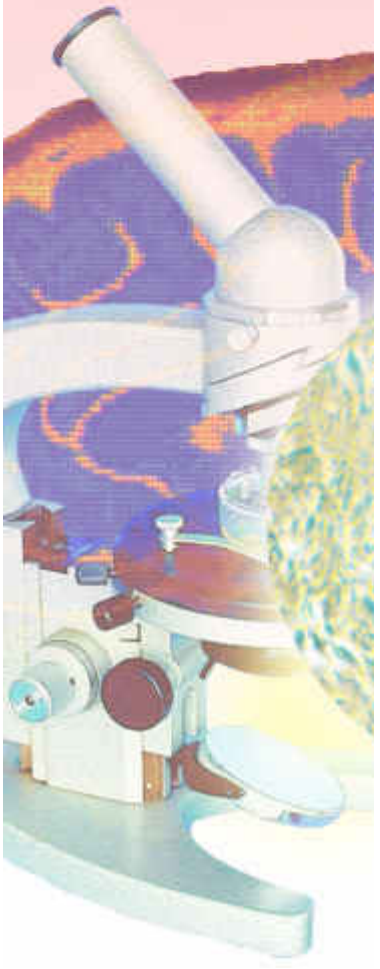
r) solving of some problems of the university community in institutions for high level medical education;

s) granting of scholarships for studies and research for teaching and research personnel or to students in high level medical education institutions, scholarships which will support high performance students and teaching personnel, their progress, specialization, participation to scientific research, information and study fellowships in Romania and abroad;

t) editing and/or supporting the material expenses for editing publications, scientific or artistic works in the fields of interest of the Foundation as well as establishing and financing, according to the law, of a publishing house / printing house for achieving this objective.

The objects of „Ana Aslan“ International Academy of Aging are:

- Publication, support and promotion of scientific works and printed materials, including „Brain Aging“ International Journal
- initiation, organization and support of projects and programmes for people of age in the scientific, cultural, educational and communication fields;
- promoting the implementation in the system of services provided for people of age of the the recommendations of the World Assembly of People of Age, of the European Community and of the recommendation expressed on scholarly level by the medicine Universities of Romania, Sweden, Greece, Israel, Thailand, Germany as well as of other countries where the Academy has established branches.
- organization and working of an excellence medical centre containing medical services and services of scientific investigations in various fields to provide for a high level of medical assistance and attendance of medical, socio-medical or psycho-affective nature for people of age who suffer from total or partial loss of their autonomy.
- initiation, organization and support for educational, research and scientific projects and programmes for the medical staff (doctors, nurses, social assistants)and promotion of innovations and their implementation in the system of the services provided for people of age;
- initiation, organization and support for courses, workshops, discussions, conferences in the interest fields of the Academy and of „Ana Aslan International“ Foundation and the promotion of co-operation between the speciality university departments of the Faculties of Medicine in Romania, Sweden, Greece, Israel, Thailand, Germany, as well as in other countries were the academy has branches.
- granting scholarships and research fellowships for students, candidates for doctor’s degree, participants in the university and postgraduate programmes, doctors, in the interest fields of the Academy and of „Ana Aslan International“ Foundation.



"Ana Aslan"
International Academy of Aging

Our task and values

The purpose of the „Ana Aslan“ International Academy of Aging, a scientific and cultural academy organized by the „Ana Aslan“ International Foundation, is to set up, organize and support projects and programs in the medical, cultural, educational and communication fields of aging.

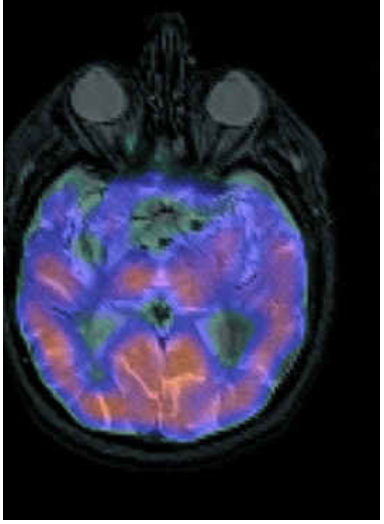
The „Ana Aslan“ International Academy of Aging focuses on health care, training, research and service system innovations that will ensure healthy aging. This includes to:

- **Enhance and expand the training of doctors, nurses, social workers and other health professionals who care for the elderly.**
- **Promote innovations in the integration and delivery of services for all elderly.**
- **Encourage and assist the development of future leaders in the field of aging, both in clinical and basic research.**
- **Expand medical research on aging through focusing on the biology of aging, diseases and disabilities of old age and clinical management issues.**

AREAS OF ACTIVITY

- Clinical Aging Research
- Epidemiological Aging Research
- Gerontological Nursing
- Physiotherapy and Occupational Therapy
- Dementia and Cognitive Disorders
- Neuroscience with focus on Molecular
- Pharmacology
- Social Science
- Health economy
- Drugs trials
- Gerontoprophilaxy

The „Ana Aslan“ International Academy of Aging will provide the hub of a network of research dealing with aging. As an active research center in itself it will also provide a rich environment where doctoral and masters students can learn from senior researchers with a wide variety of academic perspectives. Researchers from many institutions and departments will come to the „Ana Aslan“ Academy of Aging and to the Research and Education Centers so as to meet, attend courses, workshops and seminars and to give support and stimulation.



ACTIONS

Publication, support and promotion of scientific works and printed materials, including „Brain Aging“ an International Journal published by the „Ana Aslan“ International Academy of Aging.

Organization and working of medical research centers and centers of excellence containing medical services and services of scientific investigations in various fields, with the aim of providing a high level of medical, socio-medical nature for elderly people.

Initiation, organization and support for educational, research and scientific projects and programs for the medical staff (doctors, nurses, social assistants).

The First International Conference of Brain Aging – An International Journal

BUCHAREST, ROMANIA

OCTOBER 5th - 8th

THE „MARRIOTT“ HOTEL - BUCHAREST, ROMANIA

30th of June, 2003 - Abstract Submission Deadline

www.brainaging.ro

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BRAIN AGING
INTERNATIONAL JOURNAL

Why a Conference of Brain Aging - An International Journal ?

The Scientific Board of the Brain Aging International Journal has been decided to organize every year an International Meeting on Brain Aging.

Who Should Attend ?

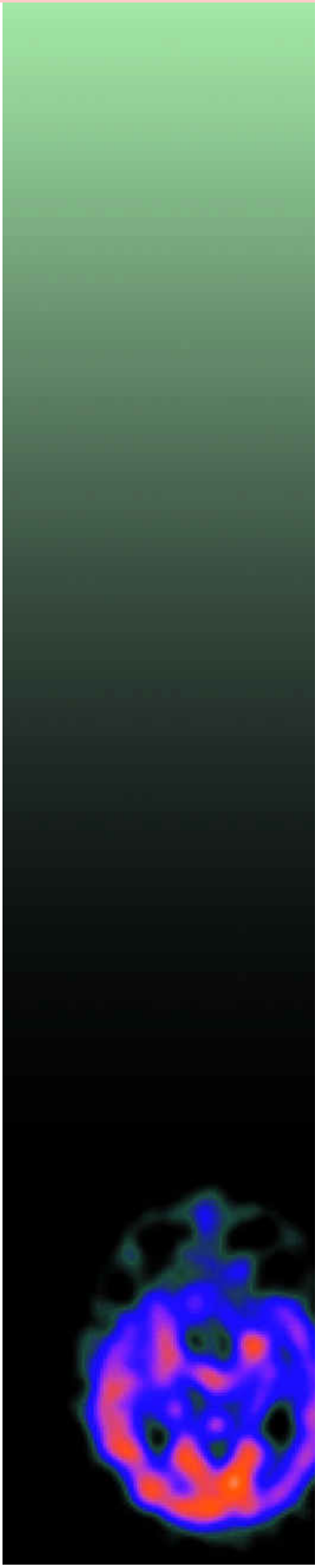
Physicians and health care workers with a special interest in the mental health and illnesses of the elderly.

These include:

Physicians in Family, General and Geriatric Medicine; Psychiatry; Neurology as well as

Nurses; Researches; Psychologists; Social workers; Program Directors; Administrators/Facilitators; Pharmacists;

and others.



„The First International Conference of Brain Aging & Aging and Dementia“

Bucharest, Romania
October 6 - 8, 2003

TOPICS:

- EPIDEMIOLOGY
- GENETICS, BIOMARKERS, NEUROIMAGING and HISTOPATHOLOGY
- BASIC MECHANISMS INVOLVED IN BRAIN AGING and DEMENTIAS
- THERAPEUTIC STRATEGIES
- DIAGNOSIS / IMAGING
- ALZHEIMER DISEASE AND NON-ALZHEIMER'S DISEASE DEMENTIAS

TIME TABLE, REGISTRATION FEES AND DEADLINES

The Conference will be preceded by a one-day Training Course for Romanian Physicians (sponsored by Novartis, Romania)

REGISTRATION FEES:

Course Attendees, special rate (includes Conference) $ 345

Conference Attendees:

Academia	$ 345
Industry	$ 485
Students	$ 200
Accompanying person	$ 120

Deadline for abstracts: June 30th, 2003

BRAIN AGING
INTERNATIONAL JOURNAL

PRECONFERENCE TRAINING COURSE ON BRAIN AGING

OCTOBER 4, 2003	Registration and Reception for Course Participants and faculty		
OCTOBER 5, 2003	9:00 - 13:00	**Laura Fratiglioni**	Epidemiology and Risk Factors
		Bengt Winblad	Treatment Strategies of Cognitive Impairments
		Bruno Vellas	Nutritional Aspects on AD Patients
		Kurt Jellinger	Cerebrovascular Pathology and Alzheimer's Disease
		Khalid Iqbal	Neurobiology of Brain Aging and Dementias
	15:00 - 19:00	**„MCI - DIAGNOSIS AND DEFINITION TO TREATMENT“**	
		Round Table Discussions:	
		Round Table 1:	Risk Factors and Diagnosis
		Round Table 2:	Therapeutics
		Round Table 3:	Neuropathology and Molecular Pathology

THE CONFERENCE: October 6 - 8, 2003

Preliminary Program

1. EPIDEMIOLOGY	**Laura Fratiglioni**	„Cognitive Impairment and Dementia in Old People"
	Gordon Wilcock	„Prediction of Conversion of Questionable Dementia to Dementia"
	Lon S. Schneider	„Current Clinical Trials and Methodology"
2. GENETICS, BIOMARKERS, NEUROIMAGING and HISTOPATHOLOGY	**Harold Hampel**	„Evaluation of Phosphorylated Protein as a Biological Marker of Alzheimer's Disease"
	Patrizia Mecocci	„Biological Markers in Dementia"
	Nicholas Sergeant	„Alzheimer Disease: A True Tauopathy Fueled by APP Dysfunction"
Relevance	**Remi Quirion**	„Key Phenotypes of the Alzheimer's Brain - Possible to Functions"
	Hilkka Soininen	„Risk Genes of Alzheimer's Disease"
	Kurt Jellinger	„The Impact of Cerebrovascular Pathology in Alzheimer Disease"
	Giulio Maria Pasinetti	„Protein microarray and clinical diagnostic in mild cognitive impairment"
	Lars Backman	„The Role of Dopamine Functions in Cognitive Aging"
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Detection of mRNA Level and Mutation of Neurofilament in Alzheimer Disease

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Abstract:

We have found recently that in addition to hyperphosphorylation, the level of neurofilament (NF) protein is significantly increased in Alzheimer disease (AD) brain. To explore the mechanism leading to this augmentation, we have detected in the present study the level of NF-M and NF-L mRNA by semi-quantitative reverse transcription PCR, and any tentative mutation of NF-M and NF-L by single strand DNA conformation polymorphism technique. The results showed that the level of NF-M and NF-L mRNA was significantly lower in AD than that of age-matched Huntington disease (HD) patients. Additionally, the mutation in NF-M but not in NF-L was detected in one case of AD patient. However most AD patients did not contain NF mutation, suggesting that NF mutation at most only partially contributes to the abnormality of NF found in minority of the AD patients. The data suggested that the increased neurofilament protein we have observed previously in AD brain is neither due to its increased gene transcription nor to its gene mutation.

Keywords: Alzheimer disease; Neurofilament; Mutation

Introduction

Alzheimer disease (AD) is the most common cause of dementia in adults. A pathological hallmark of AD is the disruption of the neuronal cytoskeleton. It is known that abnormal phosphorylation of microtubule associated protein tau is involved in such process. Neurofilament (NF) is a major component of neuronal cytoskeleton and located in the same cellular compartment as microtubules and tau. NF is composed of three protein subunits with apparent molecular masses of 200 kDa (NF-H), 140-160 kDa (NF-M), and 68-70 kDa (NF-L). In early 1980s, Sternberger's group found by using immunochemistry technique that NF was hyperphosphorylated in AD brain[1]. However, because of the cross-reaction of NF antibodies with abnormally phosphorylated tau, the results were uncertain. In our previous study, we demonstrated that NF was indeed hyperphosphorylated in AD brain by Western blot, which was able to discriminate tau cross reaction by its significantly smaller molecular weight than neurofilament subunits. In addition to hyperphosphorylation, we also found that the level of all three NF subunits, especially NF-M and NF-L was significantly increased in AD brain[2]. To further explore if the increase of NF-M and NF-L was a result of the enhancement of transcription, we used semi-quantitative RT-PCR in the present study to detect mRNA level of NF-M and NF-L in AD brain. We found that phosphorylated NFs in AD appeared to be localized mainly in perikaryon and proximal axon, which resembled the intracellular distribution of NF in another neurodegenerative disease—amyotrophic lateral sclerosis (ALS)[3]. Because NF mutation was reported in some sporadic ALS patients, and transgenic animal model expressing the mutated NF developed similar pathologic changes[4], we also investigated if there was any mutation in AD patients by single strand DNA conformation polymorphism (SSCP).

Materials and Methods

Brain samples and reagents

For this study neocortex from 8 AD and as controls 7 age-matched Huntington disease (HD) cases was employed. The brains had been removed 3-6 h post mortem and were stored frozen at –75ºC until used. The diagnosis for AD

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cases was histopathologically confirmed. For HD, the diagnosis was made based on both family history and neuropathological examination. Autopsied brain specimens were obtained from the Brain Tissue Resource Center (Public Health Service Grant MH/NS 31862), McLean Hospital, Belmont, MA, USA, and from Institute for Basic Research Brain Bank (Dr. Piotr Kozlowski).

DNA extraction kit was from CloneTech (USA). Trizol kit, one step RT-PCR kit, primers of NF-M, NF-L and GAPDH were from Gibco. The primer sequence of GAPDH was 5' ACC ACC ATG GAG AAG GCT GG 3', 5'CTC AGT GTA GCC CAG CAT GC 3'.The primer sequence of NF-M and NF-L is shown in Table 1. For each pair of oligonucleotides the sense primer is located upstream of an intron. The corresponding antisense primer is located downstream of the intron. Accordingly, depending on the size of the resulting PCR product, it can be concluded that the product is derived from the first strand cDNA. PCR products derived from potential contaminants of template with genomic DNA can be distinguished by their greater size on agarose gel electrophoresis.

Table 1. Strategy for detection of specific transcripts encoding NF-M and NF-L

Primer	Orientation	PCR product	Nucleotide sequence[5-6]
NF-M	Sense	309bp	5' tcgtcatttgcgcgaatacc 3'
NF-M	Antisense		5' gccaattcctctgtaatggc 3'
NF-L	Sense	537bp	5' agtggaggaaaccattgagg 3'
NF-L	Antisense		5' ttgccgtagatcctgaactc 3'

Total RNA extraction from human brain tissue

Total RNA was prepared from cryopreserved human brain specimens using Trizol kit according to manufacturer's instruction. RNA was quantitated by absorbance ratio of 260nm/280nm. To ensure the purity of RNA, only the samples with the ratio value of 1.8 or higher were selected for further studies. The integrity of RNA was further determined by observing 28S and 18S RNA bands separated by 1.3 % agarose gel and visualized under UV light.

Semi-quantitative RT-PCR analysis

RT-PCR was performed using one step RT-PCR kit according to manufacturer's instructions (Cat. 10928-018, GIBICO BRL). In brief, a total 50 µl RT-PCR reaction mixture contains 0.3 µg RNA, 200 µmol/L dNTPs, 0.2 µmol/L primer, 1.2 mmol/L Mg^{2+}, and 1 µl of RT/Taq enzymes. RT-PCR amplification was programmed as follow: reverse transcribed at 50°C for 30 min and pre-denatured at 94°C for 2min. Then, PCR amplification was carried out at 94°C for 15s, 55°C for 30s and 72°C for 55s. After 35 cycles for NF-M and NF-L and 25 cycles for GAPDH, the reaction was continued at 72°C for 10min and chilled on ice. Finally, 3µl of PCR products were isolated by 1.3% agarose gel. The gel was developed with Kodak Digital Science 1D system and quantitated by ImageProplus program.

Detection of mutation

Single strand DNA conformation polymorphism (SSCP) analysis was utilized to detect mutation of the NF-M and NF-L gene. PCR products were denatured by heating at 95°C for 5 min in denaturing buffer containing 95% formamide and 10 mM EDTA, and then chilled on ice immediately. The denatured products were loaded onto 6% PAGE gel (containing 10% glycerol) and electrophoresed at 50V, 4°C for 6 h. Then PCR products which showed abnormal bands were purified using DNA extraction kit according to manufacturer's instructions. The purified PCR products were sequenced by Takara Biotech (Japan). All experiments were repeated at least for three times.

Statistical analysis

Student's t test was used for all statistic analysis. The quantitative analysis was performed by using housekeeping GAPDH gene as an internal control.

Results

Total RNA extraction from cryopreserved human specimen

As RNA is biochemically unstable and is easily degraded during post mortem preservation, we first tried out if we were able to obtain intact RNA for further study. The results showed that both 28S and 18S RNA bands were clearly seen in samples prepared from human post mortem brain (Fig 1), suggesting that the quality of the RNA was good enough for RT-PCR analysis.

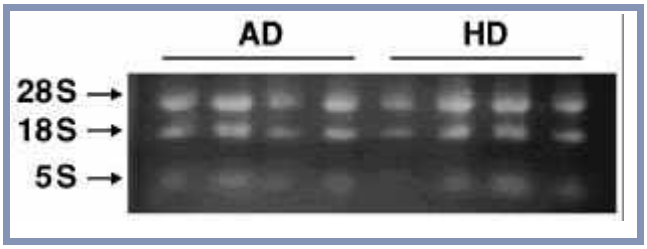

Fig 1. Identification of the integrity of total RNA extracted from cryopreserved human specimen

Table 2. Level of NF-L and NF-M mRNA detected by RT-PCR (OD_{NF-M}/OD_{GAPDH} or OD_{NF-L}/OD_{GAPDH} X ±SD)

	AD	HD
NF-M	0.30 ± 0.03*	0.42 ± 0.07
NF-L	0.44 ± 0.16*	0.79 ± 0.09

* P<0.01 vs HD

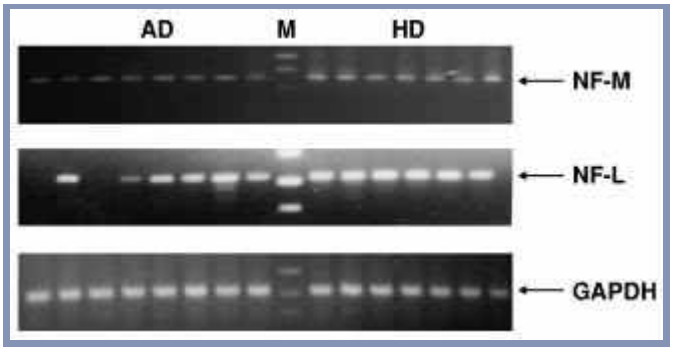

Fig 2. RT-PCR products of NF-M, NF-L and GAPDH. M: PCR marker

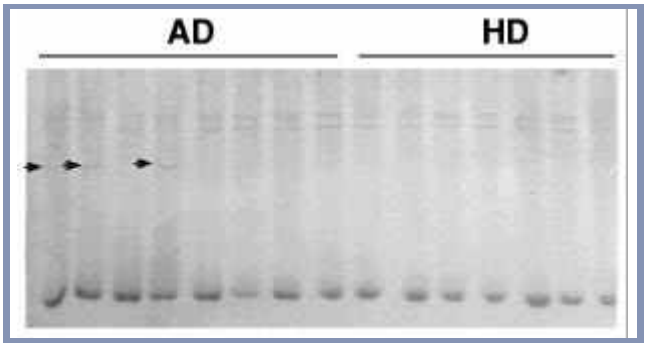

Fig 3. Silver stain of NF-M PCR product analyzed by SSCP (Arrow head shown the abnormal bands)

a TGTCAGGGCCT CTTCCATTTC TGACTTCTCA TCCTCCACTT TGGTTTCCTC TATGATCTCC TCGACAAATT
b TG CAGGGCCT CTTCCATTTC TGACTTCTCA TCCTCCACTT TGGTTTCCTC TATGATCTCC TCGACAAATT
a TGTGTTGGAC CTTAAGCTTG GGAGCTTCCA CCTTGGTTTT CTGAATCTTA CTGGATATTG TGATTGGGGG
b TGTGTTGGAC CTTAAGCTTG GGAGCTTCCA CCTTGGTTTT CTGAATCTTA CTGGATATTG TGATTGGGGG
a TCGGTGTGGTA TACAGTGGCC CAGTATGCT TCCTGCAAAT GTGCTAAATC TAGTCTCTTC ACCCTCCAGG
b TCGGTGTGGTA TACAGTGGCC CAGTATGCT TCCTGCAAAT GTGCTAAATC TAGTCTCTTC ACCCTCCAGG
a AGTTTTCTGT ACGCAGCGAT TTCTATATCC AGAGCCATCT TGACGTTGAG GAGGTCCTGG TATTCGCGCA
b AGTTTTCTGT ACGCAGCGAT TTCTATATCC AGAGCCATCT TGACGTTGAG GAGGTCCTGG TATTCGCGCA
a AATGACGA
b AATGACGA

Fig 4. Sequence of normal (a) and mutated (b) NF-M in AD patients

Detection of NF-M and NF-L RNA level

Then, we detected the level of NF-M and NF-L mRNA from AD and HD brain by semi-quantitative RT-PCR. It was found that the mRNA level of NF-L and NF-M was significantly lower in AD than HD patients ($p < 0.01$) (Fig 2 and Table 2). We also found that NF-L mRNA was not detected in some of the AD cases. To exclude the possibility of RNA degradation during the process, we assessed the internal control GAPDH mRNA, and found no difference between AD and HD samples (Fig 2), indicating that the decrease or disappearance of NF mRNA in AD cases was specific and not due to some non-specific degradation.

Detection of mutation of NF-L and NF-M

By using single strand DNA conformation polymorphism (SSCP) technique, we found abnormal bands of NF-M in three AD cases among all the AD and control patients (Fig 3). The samples with abnormal bands were then purified and analyzed by auto-sequencing. We found a deletion mutation in one of the three AD patients (Fig 4). On the other hand, no abnormal band was observed with NF-L in all the samples examined (not shown).

Discussion

We have previously found that NF protein subunits were hyperphosphorylated, and the total levels of NF-H, NF-M and NF-L were significantly increased in gray matter of AD brain[2]. To explore the mechanism that might be involved in this abnormal elevation, we investigated, in the present study, the level of NF mRNA by using semi-quantitative RT-PCR. To our surprise, it was found that the level of NF-L and NF-M mRNA significantly decreased in AD brain gray matter. To analyze the possible reasons for a decreased mRNA found in AD brain, we looked for any mutation in NF. A previous study has shown that that the 3'-untranslated region is a key domain that determines the stability of NF-L mRNA and mutations within this domain significantly decrease the stability of the mRNA[8]. Therefore, we chose to amplify this domain of NF-L, but found no mutation in this domain, indicating that the decreased stability of mRNA caused by gene mutation was not involved in decreased level of NF-L mRNA in AD brain. It should be pointed out that the domain amplified by RT-PCR was not detected in two AD and one HD patients. The reason for this anomaly is currently not understood. Further investigation of possible mutations within primer binding domain may be useful to explain the question. Additionally, a feedback inhibition in protein synthesis caused by accumulation of NF proteins detected both in cerebrospinal fluid[9] and brain tissue[2] might also contribute to the decreased mRNA level.

As mentioned above, NF protein subunits were elevated in AD patients[2, 9]. Biochemically, the possible mechanism for an increased protein level accompanied by a decreased mRNA content found in AD brain might be explained as follows: (1) Increased protein synthesis: NF is a main component of the neuronal cytoskeleton, which plays an important role in keeping the integrity of cytoskeleton, axonal transport and determining the axonal diameter. Because of an increased cytoskeletal disruption and neurodegeneration in AD brain, the neuronal cell might start a faster translational mechanism as a protective compensatory response, and thereby, lead to increased synthesis of NF proteins. (2) Decreased protein degradation: Normally, NF subunits are synthesized in cell body and degraded during their transportation to axonal terminal. In AD patients, NF is abnormally hyperphosphorylated, which on one hand might result in decreased transportation velocity[10] and decreased degradation of NF in axonal terminal. The hyperphosphorylation of NF might lead to a reduced calpain-mediated proteolysis[11], which in turn could induce a decreased degradation and accumulation of NF in cell body. The latter might be the key reason for the increased level of NF proteins found in AD brain.

As RNA is easily degraded, we were not very sure if we could obtain RNA with good quality from cryopreserved human brain for the study. The results demonstrated that proper preservation of brain samples caused no obvious degradation of RNA. It was also reported previously that the RNA analysis could be made if the brains were removed shortly after post mortem and promptly stored frozen at –75ºC[7].

We have also found for the first time in one AD patient a deletion mutation in NF-M. Previously, NF mutation was reported in some sporadic ALS patients and the mutation could induce accumulation of NF in cell body and perikaryon[3]. The present study demonstrated that in most AD patients the increased level and phosphorylation of NF was probably due to decrease in degradation and not mutations.

Acknowledgement

We thank sincerely to Drs. K Iqbal and I Grundke-Iqbal in NYS Institute for Basic Research, Staten Island, New York, USA for their years selfless scientific direction, patient correction of the manuscript and financial support in providing precious samples and reagents. Thanks to Janet Biegelson for her help in editing the manuscript. This work was supported in part by grants 39925012, G1999054007, 39870767 from the Natural Science Foundation of China, Science and Technology Committee of China and National Educational Committee of China.

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Activation of p38 Mitogen-activated Protein Kinase in 139A or 22A Scrapie-infected SAMP8, SAMR1, AKR and C57BL Mice

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Abstract

Neuropathological examination shows apoptosis and astrocytosis in scrapie-infected animals. In other studies, it has been reported that the p38 mitogen-activated protein kinase (p38 MAPK) is responsible for transduction of inflammatory signals and for initiating apoptosis. Using immunocytochemical methods, we investigated p38 activation in the brains of SAMP8, SAMR1, AKR and C57BL mice infected with the 139A or 22A scrapie strain and compared the results to those in mice injected with normal mouse brain homogenate (NMB). In 139A or 22A scrapie-infected brains, increased levels of phosphorylated (activated) p38 were detected relative to NMB-injected mice. Intense phospho-p38 immunoreactivity was detected in neurons and glial cells in cortex, hippocampus, thalamus, hypothalamus, amygdala and cerebellum. Since p38 causes increased expression of cytokines that are involved in inflammation, our results suggest that there is a p38-related innate inflammatory mechanism in scrapie brain. We hypothesize that during scrapie infection prion protein can stimulate production of cytokines by different cell types and that the neuronal apoptosis might be due to a "cytokine snowball effect" activated by p38 pathways.

Keywords: Scrapie, Neuropathology, SAMP8, p38 MAKP, Glial cells, Immunocytochemistry.

Introduction

Scrapie is the archetypal neurodegenerative disease and is found naturally in sheep and goats[1]. Scrapie-like diseases are found in other animals; the diseases include bovine and feline spongiform encephalopathy, transmissible mink encephalopathy, and chronic wasting disease in mule deer and elk. In humans, the diseases are kuru, Creutzfeldt-Jakob disease (CJD), new variant CJD, fatal familial insomnia and Gerstmann-Straussler syndrome (GSS)[2] . These diseases are sporadic, can be either inherited or transmissible and are often characterized by dementia with ataxia. Neuropathological examination shows widespread spongiform changes, prion protein (PrP^{Sc}) deposits, gliosis, and neuronal and neurite degeneration[3] .

A series of inbred mouse strains was derived from an inadvertent cross between AKR mice and an unknown mouse strain or strains[4] . These mouse strains were termed senescence-accelerated mouse prone (SAMP). Other strains derived from the same cross are resistant to early senescence, and they are termed SAMR. One of the senescence prone strains, SAMP8, shows early deficits in learning and memory, has generalized signs of aging and shows brain histopathological changes, including vacuolation of neurons and neuropil, astrocytosis, cortical atrophy, blood-brain barrier changes and alteration of hippocampal dendritic spines[5] . These changes in brain are similar to, but less intense than, those seen in strains of mice other than SAM that are infected with scrapie[6-8] . The accumulations of abnormal prion protein or amyloid plaques are sites of degenerating neurons and numerous reactive microglia and astrocytes [9,10]. Moreover, the plaques contain acute phase proteins, such as cytokines, complement proteins, proteases and protease inhibitors that are secreted by microglia[11-12] or astrocytes[9,13]. In peripheral and central nervous system, the production of inflammatory mediators such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) and acute phase proteins is

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regulated mainly by nuclear factor Kappa B (NF-κB). NF-κB binding sites in the promoters of these proinflammatory genes serve as inducible transcriptional regulatory elements[14]. However, the signal pathways involved in the neuropathological changes seen in scrapie-infected animals are still unknown.

The p38 pathway is one of the four that have been identified in mammals so far. Four isoforms, p38α, p38β, p38γ and p38δ of the p38 subfamily have been cloned and characterized[15]. It had been proposed that activation of p38 affects cellular processes including cell growth, cell cycle changes and apoptosis, and also is involved in the regulation of inflammation and stress responses[16,17]. A recently published paper showed that the stimulation of inducible NOS by the prion protein fragment 106-126 in human microglia is TNFα-dependent and involved p38 MAPK[18]. In the present study, we investigated the participation of p38 in scrapie pathology. The results reveal that the p38 pathway is activated and may play an important role in scrapie-induced changes seen in the infected brain.

Materials and methods

Animals

The SAMP8/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 8 years as inbred strains. AKR/J and C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbour, ME, USA). The animals were maintained in rooms in which the temperature, humidity, and light cycle (12 hr on, 12 hr off) were automatically controlled. The mice were given food and water *ad libitum*. The experiments were approved by the Institutional Animal Care & Use Committee in our Institute.

Scrapie Strains and Injection Procedures

The 22A-scrapie strain was obtained from Alan Dickinson (Neuropathogenesis Unit, Edinburgh, UK) and the 139A scrapie strain was obtained from Richard Kimberlin (Neuropathogenesis Unit, Edinburgh, UK). The 139A strain was maintained by passages in C57BL mice using intracerebral (i.c.) injection. The 22A strain was maintained by i.c. infection of IM/Dk mice. SAMP8, SAMR1, AKR or C57BL mice, 1.5 to 2 months old, were injected i.c. with 25 µl of 1% brain homogenate prepared from mice infected with one of the scrapie strains or with homogenate from mice injected with normal mouse brain (NMB). The mice infected with scrapie received approximately 10^6 infectious units per mouse. Each group contained at least seven animals. The characteristics of the scrapie strains have been documented previously[19-21].

Tissue Samples

Mice were killed 2 weeks after the onset of unequivocal clinical signs. The animals were anaesthetized with pentobarbital sodium (50 mg/ml, Abbott Laboratories, North Chicago, IL, USA), injected intraperitoneally at a dose rate of 3-4 ml/kg body weight. Anaesthetized animals were perfused via the heart with normal saline (15 ml/min) at room temperature for 2-3 min, followed by perfusion with 4% paraformaldehyde in 0.1 M PBS (pH 7.4, 15 ml/min) for 15 min at room temperature. Brains were removed immediately and immersed for 24 h in the paraformaldehyde perfusion solution at 4ºC. The tissues were then placed in 0.1 M PBS (pH 7.4) for 2-3 days and rinsed thoroughly. Brains were dehydrated in graded alcohols and xylene and then embedded in paraffin wax. Serial coronal sections (6 µm) were mounted on gelatin-coated slides and allowed to dry overnight at 37ºC.

Immunocytochemistry

The sections were dewaxed in xylene, placed in 100% ethanol, and then in 1% H_2O_2 in methanol for 10 min to block endogenous peroxidase activity. The sections were then rehydrated through graded ethanol solutions, rinsed in PBS, incubated for 30 min with normal sheep serum (Cappel Organon Teknika Co., Durham, NC, USA) diluted 1:50 in PBS, and then incubated overnight at 4ºC with primary antibody. Polyclonal antisera reacting with phospho-p38 (Threonine180/Tyrosine182) (1:1000, #9211S, New England BioLabs, Inc., Bevery, MA) and polyclonal antisera against GFAP (1:500, BioGenex, San Ramon, CA) were used.

Immunostaining was accomplished using either the peroxidase-antiperoxidase (PAP) or the alkaline phosphatase (BioGenex, San Ramon, CA) technique. The immunocytochemistry methods were described previously[10]. Some of the slides were counterstained with cresyl violet.

Results

The p38 phosphorylation state was assessed in different brain areas from control and scrapie-infected C57BL, SAMP8, SAMR1 and AKR mice. Immunocytochemical analysis of phospho-p38 was performed using a polyclonal antibody purified by protein A and peptide affinity chromatography. This phospho-p38 antibody detects phosphorylated Threonine 180 and Tyrosine 182 of p38 MAP Kinase (dual phosphorylated). But it does not appreciably cross react with the corresponding phosphorylated forms of either p42/44 MAPK or stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK) MAPK[22, 23]. This antibody has been used to detect phospho-p38 in brains of Alzheimer's disease (AD)

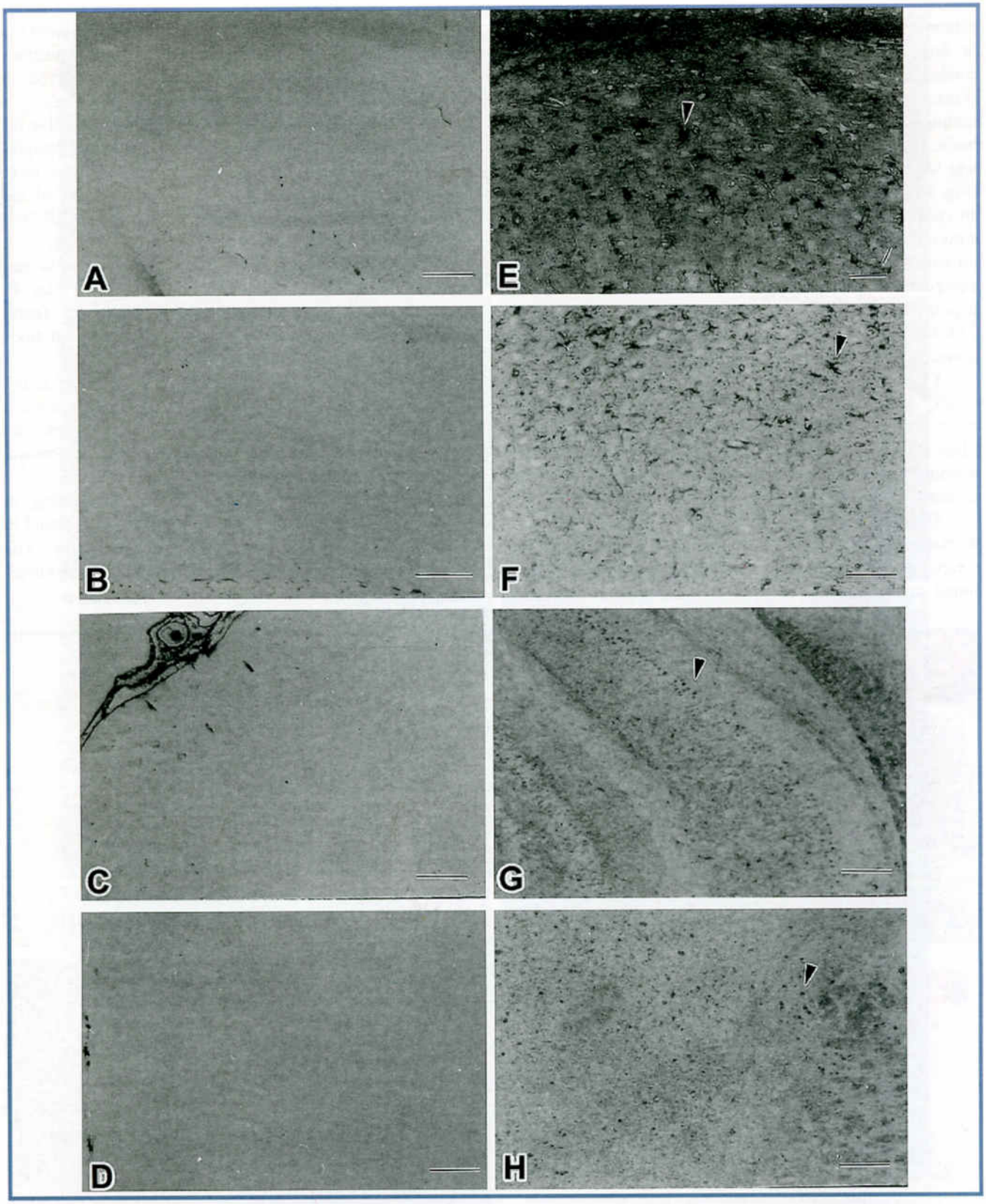

Figure 1. **(A-C).** no phospho-p38 immunostaining neurons were found in NMB-injected SAMR1 mice, magnification bar = 200 μm, **A.** cortex, **B.** hippocampus, **C.** thalamus; **D.** no phospho-p38 immunostaining neurons were found in the cortex of NMB-injected SAMP8 mice, magnification bar = 200 μm; **E.** phospho-p38 immunostaining astrocytes (arrowhead) in 139A-infected AKR mice, magnification bar = 100 μm; **F.** GFAP immunostaining astrocytes (arrowhead) in hypothalamus of 22A-infected SAMP8 mice, magnification bar = 100 μm; **(G-H).** phospho-p38 immunostaining neurons (arrowhead) in 22A-infected SAMP8 mice, magnification bar = 200 μm, **G.** hippocampus, **H.** hypothalamus.

patients [24]. In control (NMB injected) mice, either very weak or no p38 immunocytochemical staining was found in cerebral cortex, hippocampus, thalamus and hypothalamus (Figs. 1A-1D). However, in scrapie-infected mice, p38 immunocytochemical staining was found in many areas of brain. In 22A-infected SAMP8 mice, p38 immunostaining was found in different areas of brain, particularly in septum (Fig. 2A), cortex (Fig. 2B), hippocampus (Figs. 1G and 2C), amygdala (Fig. 3D), thalamus (Fig. 2D), medulla, cerebellar cortex and hypothalamus. In 22A-infected C57BL mice, p38 immunostaining was extensive throughout the cortex, amygdala, septum, hippocampus, medulla, thalamus, hypothalamus and cerebellar cortex. 139A-infected mice and 22A-infected AKR mice also showed similar p38 immunostaining patterns (Fig. 3A-3C and 3E-3G).

Cortex: No p38 immunostained cells were found in control mice. Neuronal staining was found in all layers of cortex especially layers 1 and 2 in scrapie-infected mice. Most of the p38 immunoreactivity was found within the neurites and cytoplasm; there was some staining in a few neuronal nuclei (Fig. 3B).

Hippocampus: In control mice, there were no p38 immunostained cells found in the hippocampal area. In scrapie-infected mice, most of the p38 immunostaining was found in neurons of CA1 and CA2 areas (Fig. 3H). p38 immunoreactivity was also found at the lower portion of the granular layer of the hippocampal dentate gyrus. Phospho-p38 immuno-positive astrocytes were also observed in hippocampal areas of scrapie-infected mice (Figs. 1E).

Amygdala: Numerous neuronal cells were positive for p38 immunostaining in the amygdala cortex in scrapie-infected mice. High levels of p38 immunoreactivity were also found in the basolateral and cortical regions of the amygdala in 22A-infected SAMP8, SAMR1, AKR and C57BL mice.

Corpus callosum: p38 immunostaining was not present in cells in the corpus callosum of control mice. In contrast, a few p38 immunostained cells were found throughout the corpus callosum in scrapie-infected mice (Figs. 3A).

Septum: Immunoreactivity of p38 was also found throughout the septum and fimbria of scrapie-infected mice, but not in control mice. Both neurons and non-neuronal cells were positive for p38 immunoreactivity in scrapie-infected mice in this area (Fig. 2A).

Thalamus: We found no p38 immunostaining in control brain, whereas, p38 immunoreactivity was found in neurons and glial cells in scrapie-infected mice. The staining intensity was less in the thalamus than in the cortex and hippocampus.

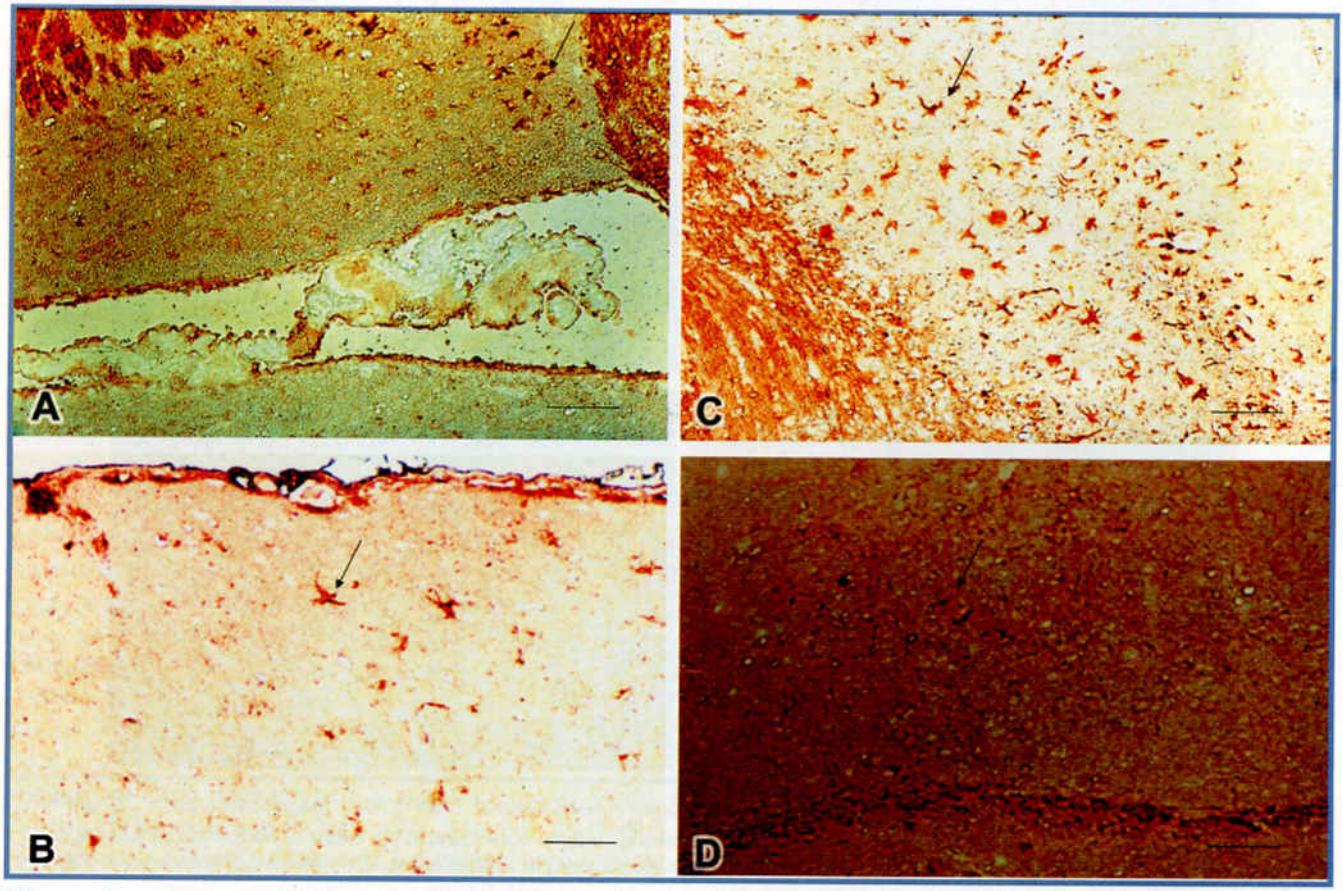

Figure 2: (A-D). phospho-p38 immunostaining astrocytes (arrowhead) in 22A-infected SAMP8 mice, magnification bar = 100 μm, **A.** septum, **B.** hippocampus, **C.** cortex, **D.** thalamus.

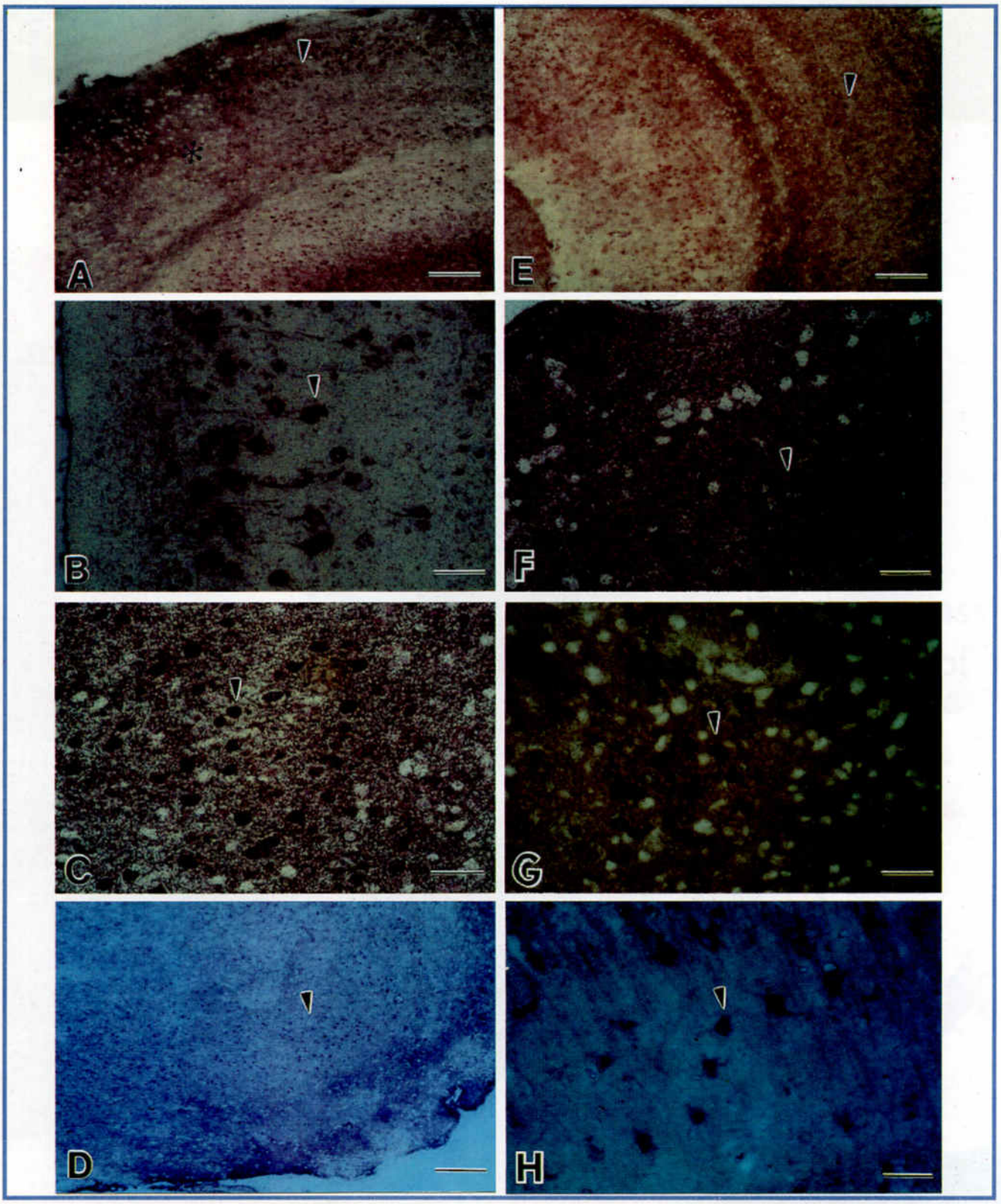

Figure 3. **(A-C).** phospho-p38 immunostaining neurons (arrowheads) and vacuolation (*) in 139A-infected SAMP8 mice, **A.** cortex, magnification bar = 200 μm; **B.** cortex, magnification bar = 50 μm; **C.** hippocampus, magnification bar = 50 μm; **(D).** phospho-p38 immunostaining of neurons in cerebellum of 22A-infected SAMP8 mouse, magnification bar = 200 μm; **(E).** phospho-p38 immunostaining neurons (arrowhead) in cortex and hippocampus of 22A-infected SAMR1 mouse, magnification bar = 200 μm; **(F).** phospho-p38 immunostaining neurons (arrowhead) and vacuolation (*) in cortex of 22A-infected C57BL mouse, magnification bar = 100 μm; **(G).** phospho-p38 immunostaining neurons (arrowhead) and vacuolation (*) in thalamus of 22A-infected AKR mouse; and **(H).** phospho-p38 immunostaining astrocytes (arrowhead) in hippocampus of 22A-infected AKR mouse, magnification bar = 50 μm.

Hypothalamus: There were no p38-positively stained cells in the hypothalamus of control mice; however, in scrapie-infected mice extensive p38-immunoreactivity was found in the paraventricle area (PVH), suprachiasmatic nucleus (SCN) and supra-optic area (SON). Some of the glial cells were also positive for phospho-p38 immunostaining.

Phospho-p38-positive cells were also found in claustrum, caudate putamen, mammillary nuclei, substantia nigra, medulla and in the layer of the granular cells of the cerebellum in scrapie-infected mice.

In this study, we observed extensive GFAP immunostaining astrocytes in scrapie-infected SAMP8, SAMR1, AKR and C57BL mice (Fig. 1F), but not in NMB-injected mice. Most GFAP-positive astrocytes were found in cortex, hippocampus, thalamus and hypothalamus. Some of the astrocytes were also p38-positive (Figs. 2A and 2C).

Discussion

The mechanism involved in apoptosis observed in scrapie-infected animals is still unknown. In a previous study, we found that c-Fos immunoreactivity was increased in scrapie-infected SAMP8, SAMR1, AKR and C57BL mice. In this study, we found that phospho-p38 immunoreactivity was also increased in these strains of mice following infection with a number of scrapie strains. Recently, increased levels of phosphorylated (activated) p38 were also detected in brains of AD patients [24]. The phospho-p38 immunoreactivity in AD brains was localized primarily in neurons within and near plaques and in glia close to plaques. Phospho-p38 immunoreactivity was also observed adjacent to dying CA1 neurons of the hippocampus after global forebrain ischemia [25]. In the current study, we observed extensive cellular vacuolation in the cerebral cortex of scrapie-infected mice. Most of the cellular vacuolation was found in layers I and II of the cortex areas, which also showed neuronal p38 immunostaining. Cellular vacuolation and p38 immunostaining can also be found in many brain areas such as the deep layer of cortex, hippocampus, septum, thalamus, hypothalamus and amygdala. This study suggests that vacuolation in scrapie may be due to neuronal apoptosis caused by activated p38 pathways.

The mechanism of p38 activation in scrapie-infected brain is not clear. The p38 kinase enzyme resides quiescently in the cytosol until activated by dual phosphorylation on a specific domain (Thr180-Gly181-Tyr182) located in the subdomain VIII [26]. MAPK kinase-3 (MKK3) and SEK (a glycosylated protein tyrosine kinase) activate p38 by phosphorylation at Thr180 and Tyr182. Activated p38 has been shown to phosphorylate and activate MAPK activated protein kinase-2 (MAPKAP kinase-2) [27]. It has also been shown that activated p38 phosphorylates and activates the transcription factor ATF-2 [26], cyclic AMP-responsive element binding protein (CREB), the DNA damage-inducible gene, monocyte enhancement factor 2C and Max (members of the Bcl-2 protein family)[28-30]. The highly specific p38 inhibitor, SB203580, completely blocked TNF-induced activation of MAPKAP kinase-2 and heat shock protein 27 (hsp27) phosphorylation[31]. p38 can also increase c-Fos, and c-Jun expression[32]. p38 phosphorylates ATF-2 at its threonine 69 and 71 residues with subsequent c-Jun gene expression mediated by the AP-1-like binding sites Jun1 and Jun2 in the c-Jun promotor[33]. p38 kinases contribute to induction of c-Fos gene via ELK-1[34], which binds with serum response transcription factors (SRF) to the serum response element (SRE) binding site of the c-Fos promotor. Moreover, c-Fos induction can also be conferred by binding of CREB to its calcium response element (CRE) consensus sequence following phosphorylation of MAPKAP-kinase 2, another substrate of p38[35]. Recently, we also reported increased c-Fos immunostaining in scrapie-infected mice. These studies suggested that the upstream and downstream of p38 pathways were activated during scrapie infectious.

Transcription factor recruitment in response to p38 activation facilitates expression of specific gene products. In particular, p38 appears necessary for IL-1 and TNF-α synthesis in monocytes, fibroblasts, and endothelial cells[14,36]. It also appears to be necessary for cyclooxygenease-2 expression in NIH 3T3 cells, fibroblasts, and endothelial cells[36,37]. Expression of inducible nitric oxide synthase (iNOS) in astrocytes and in microglia is also dependent, at least in part, on the p38 pathway [22]. Recent studies indicate that p38 is associated with caspase-1/ICE activation[38]. Caspase-1/ICE has also been immunolocalized to microglia after ischemia [39], and pro-IL-1β, a substrate of caspase-1/ICE, is secreted by activated microglia[40]. It has also been reported that activation of FAS and caspase 3 are involved in the early pathological sequence of events during 87V scrapie infection [41].

p38 phosphorylation has been reported in microglia following a stroke in a gerbil model[25] and after ischemia in heart and brain[42]. p38 phosphorylation in microglia is strongly stimulated by contact with Aβ[43]. The phospho-p38 immunoreactivity of the AD brain is localized primarily in neurons[17,44,45]. Phosphorylation of p38 is a response to cellular stress; therefore activation in scrapie-infected brain is not likely to be an initiating factor in the disease but rather a response to preexisting stimuli such as prion protein (PrP^{Sc}) or inflammatory cytokines. Prion protein is found in scrapie-infected animals and is toxic to neurons and trophic to astrocytes[46]. Recently it has been found that heat shock protein, transferrin, β2-microglobulin, IL-1, TNF-α, NF-κB, iNOS and c-Fos expression were increased in scrapie-infected brain[47-51]. Increased production of inflammatory cytokines has been reported in glial cells in scrapie-infected animals[13]. TNF-α is an endogenous pyrogen and induces production of IL-1[52]. TNF-α produces CJD-like lesions in vivo[53] and causes myelin vacuolation in experimental CJD[54].

Because astrocytes and microglia are activated in scrapie-infected animals [9,10] and p38 is activated in microglia after ischemia [25], our findings suggest that p38 is activated in astrocytes and microglia of scrapie-infected brain.

It is our hypothesis that a snowball effect exists in the p38 pathway during scrapie disease. In such a model, the scrapie form of the prion protein, PrP^{Sc}, activates microglia and astrocytes during scrapie infection. Inflammatory cytokines such as IL-1 and TNF-α produced by activated microglia or astrocytes would activate the p38 pathway. It has been found that the p38 pathway is necessary for expression of inflammatory gene products (including IL-1, TNFα, cyclooxygenase-2 and iNOS). The increased cytokine production by activated p38 would not only stimulate the cell itself, but would also activate the cells nearby, causing a self-propagating cycle of autocrine, paracrine or endocrine stimulation. These cells (microglia, astrocytes, endothelial cells, pericytes or neurons) would further produce inflammatory cytokines, and diffusible oxidants (e.g. H_2O_2 and ·NO congeners) in a p38-dependent fashion.

Our results suggest that phospho-p38 activation may play an important role in TNFα, c-Fos, and NF-κB signal transduction during scrapie infection. This p38 signal pathway may cause neuropathology including astrocytosis, neuronal apoptosis, vacuolation and glial cell inflammatory reaction.

Acknowledgments

The authors wish to thank Ms. Joanne Stocker for editing and excellent assistance in preparation of this manuscript. The authors appreciate the excellent assistance of Ms Sharon Mathier and Mr. Daniel Klitnick for graphics preparation.

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The Density of Endothelial Cells in Brain Tissue is Influenced by Gender, Age and Concomitant Brain Pathology

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Abstract

We analyzed the density of endothelial cells (EC) as a marker for micro vascular density in the brain tissue from 97 aged subjects, employing immunohistochemical techniques. We found a significant spatial difference in the extent of EC labeling both with respect to gray and white matter (gray>white, p<0.001), and also to brain region (frontal>parietal cortex, p<0.003). The density of EC labeling decreased with age significantly (p<0.03) in the white matter of females. The density of EC labeling was significantly higher in subjects with neuritic plaques compared to subjects with only vascular lesions. Our findings indicate that the density of EC labeling in the brain is gender dependent, and is influenced by the age of the patient. Furthermore, the significant differences in EC density, comparing subjects with Alzheimer disease related lesions and vascular lesions indicate that small vessel pathology might be of importance in the pathogenesis of dementia.

Keywords: Endothelial cell, Dementia, Age

Introduction

Aging is associated with many changes in the brain tissue[1] and some of these changes are considered to be the pathological indices of dementia. These include the hallmark lesions of Alzheimer's disease (AD), the neuritic plaques (NP) and neurofibrillary tangles (NFT)[1,2]. Cerebrovascular lesions represent another pathology considered to cause dementia[3].

Few reports are available concerning the aging related changes in the capillary net. Reports have indicated that both an increase[4,5], as well as a decrease[6] occurs in the microvasculature with aging. Compared to aged non-demented subjects, in AD both unchanged[5] or a decrease in microvascular density[6] have been reported.

Recent epidemiological studies[7,8] emphasizing the association between AD and vascular risk factors have raised the possibility that the brain vasculature is of importance in the pathogenesis of AD.

The objective of this study was to analyze the density of endothelial cells (EC), covering the microvasculature, in neocortex of aged subjects, and to analyze whether or not the density of endothelium was influenced by other pathologies associated with aging.

Material and methods

The material analyzed included brain samples from 97 subjects (32 males and 65 females), age at death ranging from 73 to 88 years. The clinical assessment of the patients was made by a retrospective examination of the available medical records. For the diagnosis of dementia, DSM-IIIR (1987) and NINCDS-ADRDA criteria were used[9].

At autopsy the brains were weighed, fixed in 10% buffered formalin for at least one week and cut in coronal slices. A neuropathological evaluation was carried out according to a standard procedure[10]. In brief, infarcts were evaluated on haematoxylin eosin and AD changes were evaluated on Bielschowsky silver stained slides. The NFTs and NPs were scored as described previously[11] under light microscopy with the reported score is the sum of scores in frontal, temporal and parietal cortices.

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The endothelial cells (EC) were labeled using immunohistochemical (IHC) methodology as described earlier[10] utilizing a monoclonal antibody directed to human alveolar macrophages, HaM 56 (DAKO M632) at a dilution of 1:100. Specimens taken from three defined cortical (frontal, temporal, parietal cortices) regions from the right hemisphere were analyzed. The extent of EC labeling was estimated on 5 (white matter) or 7 (gray matter) by chance selected fields, under light microscopy, at x40 magnification, by means of the morphometrical program Quantimet 570 Image Analysis System (Cambridge Instruments, Ltd., England) and the results are given as stained area fraction (fEC).

SSPS program for Windows was used for statistical analysis including the Student's t-test and ANOVA repeated-measures analysis. The correlation between different variables was tested by means of Pearson's correlations test.

Results

Most of the subjects were females (67%) and the mean age at death was 81 ± 0.4 years. Thirty seven percent of the subjects were clinically demented. The age at death was significantly higher, whereas the MMSE and the brain weight were significantly lower in demented compared to non-demented individuals (Table 1).

Microscopic and/or macroscopic infarcts were seen in 55% of the subjects (demented/non-demented = 58% / 53%). Thirty six percent of the subjects in both demented and non-demented groups had both microscopic and macroscopic lesions, whereas only microscopic vascular lesions were seen more often in demented subjects (demented/non-demented = 22%/16%). NPs were seen in 69 % of the subjects (demented/non-demented = 83%/61%) and NFTs were detected in 29% of the subjects (demented/not-demented 58%/11%. Concomitant vascular and AD pathology, was seen in 39 (40%) out of 97 subjects (demented/non-demented = 44% / 38%).

Endothelial cell density reported as stained area fraction (fEC) (frontal+temporal+parietal cortices) was significantly ($p<0.001$) higher in gray matter (GM) (0.013±0.0005) than in white matter (WM) (0.009 ±0.0004). Similar results were obtained when the analysis was performed for each region separately. A comparison between the cortical regions revealed that both in GM and WM the fEC was lowest in parietal (GM/WM = 0.012 ±0.0005 / 0.007 ±0.0004) and highest in frontal (GM/WM = 0.014 ±0.0007/ 0.009 ±0.0005) region (GM/WM = $p< 0.003$ / $p<0.0001$).

Fourteen subjects, 7 males and 7 females, mean age at death 81 +/- 3, age range 76 to 87 years lacked macro- or micro- infarcts, and did not display AD pathology. In these control cases the changes in fEC in neocortical GM and WM were synchronous ($r=0.8$, $p<0.05$). With aging the fEC decreased in both GM and WM, with this decrease being significant in females ($r= -0.3$, $p<0.03$) in white matter. A higher value of fEC, however not significant, was found in both GM and WM of males compared to females.

No significant relationship was found between MMSE, brain weight and the extent of fEC in GM or WM.

No significant differences were found in fEC comparing subjects with and without vascular pathology, nor did the extent of vascular lesions significantly influence the fEC.

A comparison of subjects with and without AD pathology detected no significant differences in fEC in GM or WM. The counts of NP's and NFT's did not reveal any significant correlation with the fEC (Figure 1a). However, when comparing subjects with various degrees of AD

Table 1 Clinical information

Clinical diagnosis	n	Gender F/M	Age at death m±2SE	Age at onset m±SE	MMSE m±2SE (n)	BW m±2SE
Non-demented	61	38/23	80 ± 0.4[1]		26.5 ± 0.3 (38)[3]	1295 ± 23[4]
All demented:	36	27/ 9	82 ± 0.7[1]	74 ± 1.1	10.3 ± 1.9 (18)[3]	1163 ± 29[4]
AD/poAD	21	15/ 6	81± 0.7[2]	74 ± 1.1	9.7 ± 2.0 (15)	1181 ± 34
VaD	7	7/ -	85 ± 0.5[2]	78 ± 2.0	20.0 ± 2.0 (2)	1027 ± 55
Others	8	5/ 3	82 ± 2.2	71 ± 3.2	0 (1)	1237 ± 73
Σ	97	65/32	81 ± 0.4		21.3 ± 1.2 (56)	1246 ± 19

n-number of cases, F-female, M-male, MMSE-Mini mental State Examination, BW-brain weight,
(n)-number of subjects with available information, m±2SE - mean ± 2 standard error.
poAD-possible Alzheimer's disease, VaD-vascular dementia. Statistical difference when comparing the clinical groups according to Student's t-test, [1] $p<0.03$, [2,3,4] $p<0.001$.

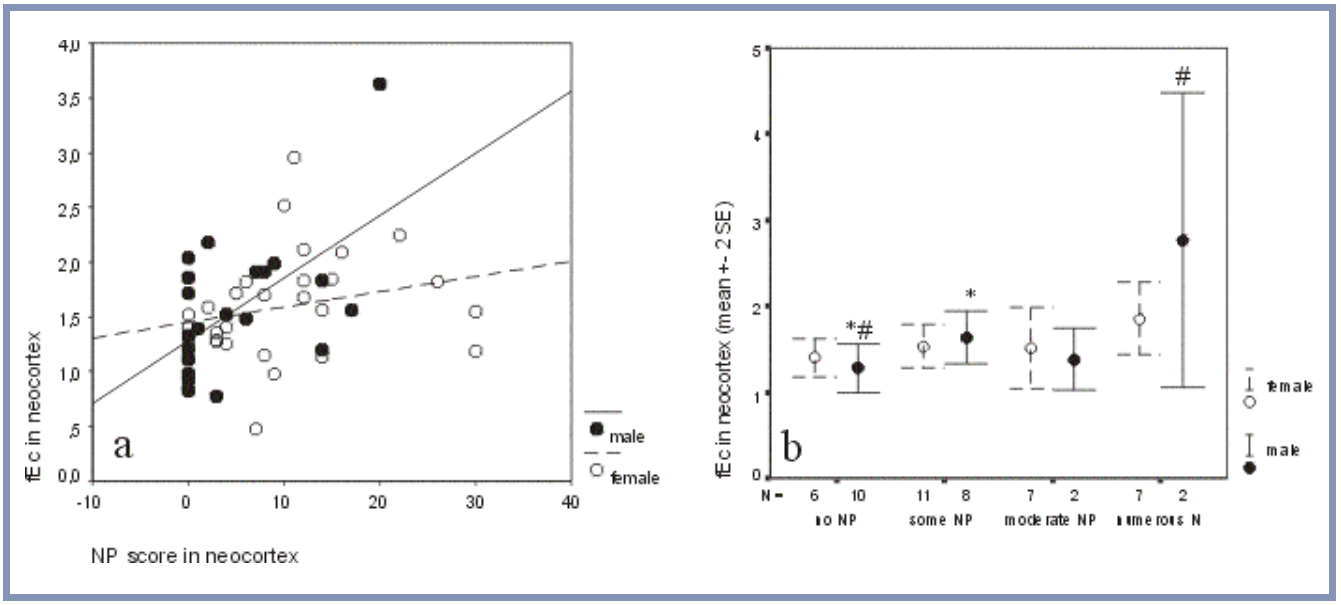

Figure 1. Endothelial cell density as stained area fraction (fEc) in gray matter using IHC NP score using BS.
a) no significant correlation (Pearsons correlation test) was found. **b)** For comparison oneway ANOVA Post Hoc LSD were used,*# p<0.005.

lesions in male subjects, a significant increase in fEC in GM was seen with addition of AD pathology (Figure 1b).

When cases were grouped according to the presence of solitary or combination of pathologies (Figure 2), it was found that when only vascular lesions were present the fEC was significantly lower and when only AD pathology was seen the fEC was significantly higher when compared to cases with no lesions.

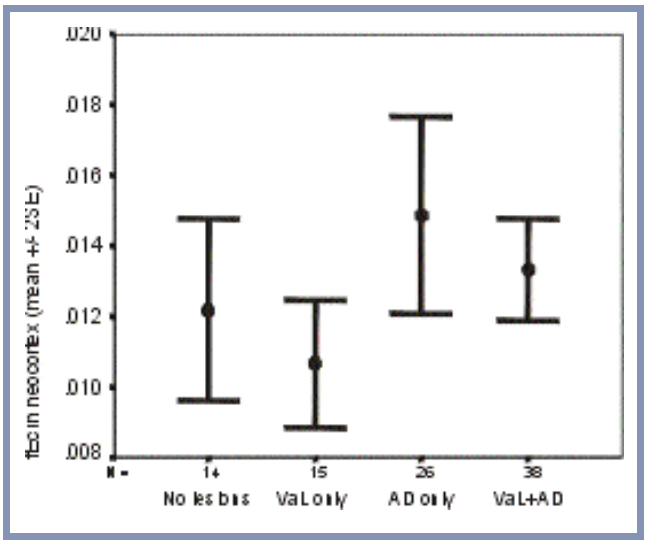

Figure 2. Endothelial cell density estimated as stained area fraction in subjects with various pathologies (VaL = vascular lesions, AD = present NP's).
For comparison oneway ANOVA Post Hoc LSD was used for comparison, p<0.05

Discussion

AD pathology was commonly seen in subjects with and without clinical signs of dementia. Overall, NP were seen in 69% of our cases which is comparable to the 70% reported by the neuropathology group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) when analyzing community based aged population in England and Wales[12]. Vascular pathology was also common in our material. Vascular pathology was present in 78% of cases in the MRC CFAS study, compared to our value of 55%. In 40% of our subjects, concomitant AD and vascular pathology was found. This is comparable to the 30% reported by Etiene et al in 1999[13] when summarizing the findings in AD cases from Massachusetts General Hospital, Alzheimer Disease Research Center, autopsy archives.

Buee and coworkers reported a decrease in vascular density[6] when comparing one 49 year old individual with three elderly subjects (79 +/- 1 year). Similar results were reported by Bell and Ball[5] when comparing five young (21-51 years) to five elderly (70-88 years) individuals. In contrast, Hunziker and co-workers[4] reported an increase in the capillary net when comparing subjects between 64 and 74 years. Our results studying the labeling of endothelial cells as a marker for vascular density indicate that there is a significant decrease in small vessel density, both in gray and white matter within the age range of 76-87 years. The decrease was however significant only in females and only in white matter. Comparable gender related results have not, to the best of our knowledge, been previously reported. The influence of gender might be one of the reasons for the

variability in previous reports. The influence of gender is interesting. Female gender is a known risk factor for dementia, and white matter changes have been implied to be of major importance in the pathophysiology of dementia[14,15]. A low small vessel density in white matter might lead to disturbances in myelin homeostasis leading to a wide range of white matter changes provoking unexpected functional disturbances, even cognitive impairment.

In 1990, Fisher and co-workers[16] analyzed the microvascular density in various brain regions in six aged control brains. According to their results, there were no significant differences between the densities in pre-frontal cortex, basal forebrain, motor sensory cortex and hippocampal formation. In our study, we did find a significant regional difference, since parietal cortex showed significantly lower small vessel densities compared to the frontal cortex. The section referred to as parietal cortex was taken from the brain area supplied by the medial cerebral artery, whereas the frontal lobe section was closest to the "watershed area". The "watershed area" is vulnerable to circulatory disturbances and this region is frequently affected with lesions in patients with cardiac dysfunction. The higher density of vessels within this region might be due to vascular proliferation, i.e. vascular plasticity, in response to circulatory dysfunction associated with aging.

In cases with cerebrovascular pathology such as notable infarcts the small vessel density was lower compared to controls. In contrast, when there was increase of NP's, the vessel density increased this being statistically significant in males. Moreover, when comparing individuals with no lesions with those with only AD lesions, the latter cases displayed a higher density of vascular profiles. Previously, both decreased vascular density[6] and increase in vascular density in AD have been reported[5].

Our results indicate that AD as well as vascular pathology are common in the aged population. The small vessel density in the brain is influenced by gender and aging and reveal regional differences. Moreover, we found a significant difference in the small vessel density in the gray matter when comparing subjects with AD lesions or vascular lesions alone. Our results indicate that small vessel changes might be of importance in the pathogenesis of Alzheimer's disease and vascular dementia.

Acknowledgements

This study was supported by the Health Research Council of the Academy of Finland and EVO grants. We thank Hannu Tiainen, Heikki Luukkonen, Tarja Kauppinen and Tarja Tuunanen for their skillful technical help.

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Interrater Reliability of the GBS Scale Used by Staff and Caregivers

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Abstract

The GBS scale, a rating scale for dementia, is used by trained staff to evaluate for the effects of interventions. The aim of the present study was to ascertain whether untrained caregivers could also use the scale. Dementia patients (n = 120) referred to a geropsychiatric diagnostic unit formed the basis of the study. Intellectual, emotional and ADL functioning and six behavioral and psychological symptoms of dementia (BPSD) were evaluated in each patient by the primary caregiver and by a trained staff member, both using the GBS scale. Interrater reliability for the 120 paired evaluations showed correlation coefficients of 0.50 for the intellectual, 0.39 for the emotional and 0.56 for the ADL subscale. The BPSD results varied somewhat more with a correlation coefficient range of 0.09-0.51. These results suggest that the GBS scale may provide a useful tool in the management of dementia patients and may be used by untrained caregiver with caution.

Keywords: Dementia, Caregiver, GBS Scale, Reliability

Introduction

The number of elderly people is steadily increasing in the Western world and with that also the number of demented people[1]. Dementia is one of the most troublesome and costly diseases. It is chronic, with slow progress and characterized by a wide range of symptoms[2]. In general the major responsibility for the daily life of a demented person rests upon the caregiver. As the disease progresses, the caregiver becomes more and more involved in the disease process and its consequences[3-5].

In order to understand dementia symptoms, the progress of the disease and to evaluate interventions the use of ratings scales is important tools. There are no reports of self-assessments carried out by demented persons, however observer and interview rating scales are reported to be widely used to evaluate patients with dementia syndromes. There are reports that describe caregivers' capability to monitor activities of daily living (ADL) functions, sleep disturbances, personality change and quality of life[6-8] in dementia. There are few reports concerning structured methods used to collect data on symptoms of dementia directly from caregivers[5].

In the care of other disorders, for example pain[9] and diabetes[10] , self-assessment tools have been used for some years. Attempts have also been made to use self-assessment in cognitive disorders such as schizophrenia. Some studies showed that schizophrenic patients were able to describe the type and degree of side effects and to identify symptoms such as cognitive impairment and depressed mood, but they had difficulties in recognizing negative and excitatory symptoms[12-14]. In child psychiatry, parents' assessment scales are used to evaluate the degree of symptoms[15].

The Gottfries-Bråne-Steen (GBS) scale[6] was developed at the Psychiatry Section of Institute of Clinical Neuroscience, Gothenburg University. It is a comprehensive, semi-structured rating scale for dementia and covers several domains. The validity of the scale is well established and its interrater reliability for health care staff is high interrater reliability. A Spearman rank coefficient of 0.87 was recorded in one study and in another study, the item demonstrated correlation coefficients between physicians and nurses 0.93 between psychologists and nurses[6]. The scale has been translated into many languages and is regularly used in clinical practice in several countries[16]. It has proven to be an appropriate tool to evaluate interventions in dementia[6]. The scale has been used in clinical caregiver support groups but no study in this respect is performed yet. The aim of the present study is to evaluate whether the GBS scale may offer a reliable and practical tool in dementia when used by untrained caregivers.

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Materials and Methods

One hundred and twenty consecutive patients with mild to moderate dementia referred to a geropsychiatric diagnostic unit in Gothenburg, Sweden, were included. Each patient was rated by a trained staff member and by his or her primary (untrained) caregiver, both using the GBS scale. Six members of the staff took part in the rating procedure. They were all nurses or a assistant nurse, had at least 4-year experience with dementia patients, all wear trained to evaluate patients with the GBS-scale and all had also the main personal responsibility for each rated patient. Thus, 126 different raters were involved in the study. The ratings by the staff and caregivers were performed independently on different occasions usually one to four days apart. The caregivers were also asked to fill in a questionnaire concerning their opinions of the rating procedure.

The GBS scale has 27 items. It is divided into four subscales assessing:

- intellectual impairment (12 items),
- emotional impairment (3 items)
- impairment of ADL performance (6 items)
- behavioral and psychological symptoms of dementia (BPSD) (6 items), this part of the scale is usually each item regarded separately.

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 symptoms. The interrater reliability for staff and caregivers was evaluated using nonparametric statistical methods. Kendall's rank correlation was used for relationships and Wilcoxon's paired rank test for differences in the levels of different ratings.

Results

The mean age of the patients (77 women and 43 men) was 73 years (range 49-88). They carried diagnoses of mild or moderate dementia . The caregivers who performed GBS ratings were spouses (64%), children (33%) or friends (3%) with different experience and background around the dementia process. The children and friends who performed the GBS-scale were main caregiver and visited the patient regular. The correlation coefficients for staff and caregiver scores were 0.50 for the intellectual subscale (Fig. 1), 0.39 for the emotional subscale (Fig. 2) and 0.56 for the ADL subscale (Fig. 3). These correlations were all statistically significant ($p<0.05$). In the BPSD subscale, five out of six symptoms showed statistically significant correlations.

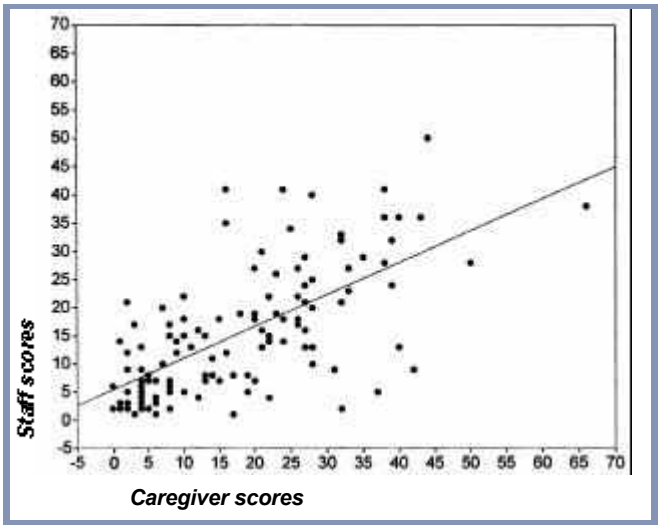

Fig. 1. Correlation between the total scores given by staff and caregivers on the intellectual subscale (r=0.50)

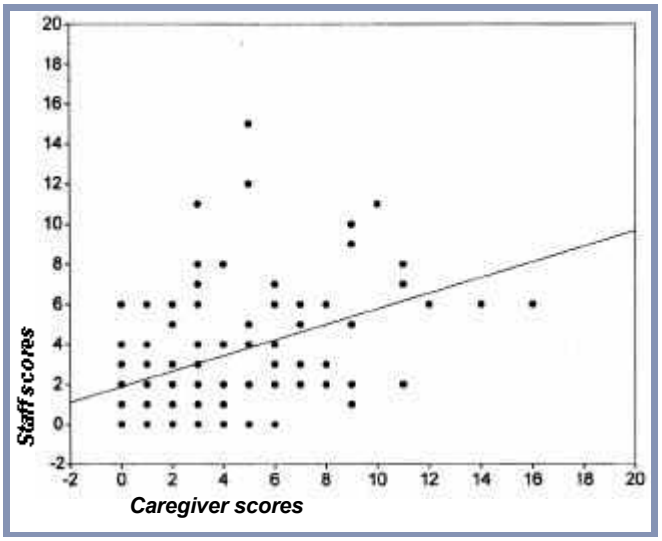

Fig. 2. Correlation between the total scores given by staff and caregivers on the emotional subscale (r=0.39)

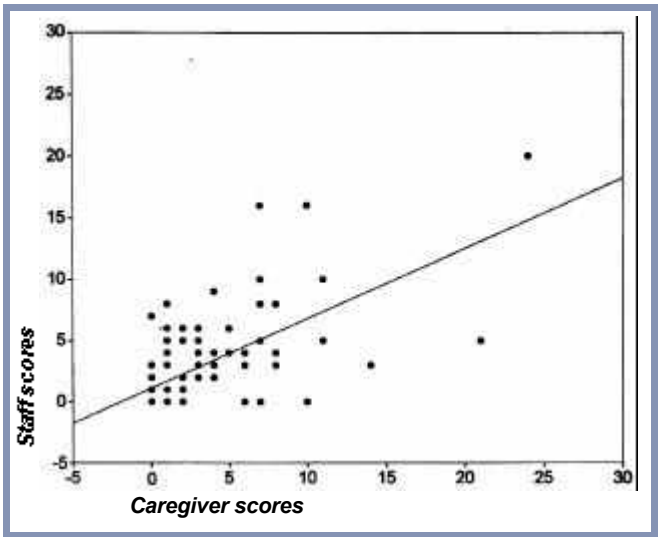

Fig. 3. Correlation between the total scores given by staff and caregivers on the ADL subscale (r=0.56)

Table 1. Scores (mean (M), standard deviation (SD), and range) given by the staff and the caregivers on all items of the GBS scale: significance of the differences and the correlations between scores.

	Staff			Caregiver			Differences	Correlation
Items	M	SD	Range	M	SD	Range	p	τ[1]
Intellectual impairment								
Orientation to person	0.72	1.039	0-4	0.48	1.004	0-5	0.01	0.49
Orientation to time	1.84	1.824	0-6	1.83	1.657	0-6	ns	0.56
Orientation to space	1.53	1.634	0-6	1.18	1.432	0-6	0.01	0.59
Recent memory	2.87	1.561	0-6	2.68	1.686	0-6	ns	0.56
Distant memory	1.93	1.403	0-6	1.39	1.311	0-6	0.001	0.46
Wakefulness	0.23	0.628	0-4	0.88	1.154	0-6	0.001	0.17
Concentration	1.33	1.231	0-4	1.74	1.498	0-6	0.01	0.42
Ability to increase tempo	1.81	1.298	0-4	2.08	1.663	0-6	0.05	0.45
Absentmindedness	1.18	1.275	0-5	1.68	1.671	0-6	0.01	0.50
Long-windedness	1.07	1.308	0-4	1.79	1.705	0-6	0.001	0.29
Distractibility	0.73	1.045	0-4	1.48	1.432	0-6	0.001	0.29
Language disturbances	0.68	1.078	0-5	0.86	1.176	0-6	0.05	0.44
Total intellectual impairment score	15.63	11.179	0-50	18.08	13.108	0-50	0.05	0.50
Emotional impairment								
Emotional functions	1.26	1.192	0-5	0.94	1.197	0-5	0.050	0.28
Emotional lability	0.76	1.037	0-5	0.73	1.158	0-6	ns	0.26
Motivation	1.28	1.283	0-5	1.95	1.905	0-6	0.001	0.40
Total emotional impairment score	3.29	2.957	0-15	3.63	3.493	0-16	ns	0.39
Impairment of ADL performance								
Dressing and undressing	0.31	0.848	0-4	0.38	0.945	0-4	ns	0.52
Meals	0.14	0.490	0-2	0.16	0.635	0-4	ns	0.27
Physical activity	0.18	0.622	0-5	0.23	0.739	0-5	ns	0.50
Spontaneous activity	0.87	1.037	0-4	0.89	1.098	0-5	ns	0.37
Personal hygiene	0.62	1.086	0-5	0.53	1.029	0-6	ns	0.66
Control of bladder and bowel	0.41	0.948	0-6	0.33	0.833	0-6	ns	0.57
Total impairment of ADL score	2.53	3.415	0-20	2.48	3.887	0-24	ns	0.56
BPSD								
Confusion	0.83	1.140	0-4	1.13	1.390	0-6	0.01	0.51
Irritability	0.36	0.838	0-5	1.36	1.302	0-6	0.001	0.22
Anxiety	1.07	1.059	0-4	2.02	1.635	0-6	0.001	0.43
Fear-panic	0.45	0.924	0-5	1.21	1.283	0-5	0.001	0.43
Depressed mood	1.30	1.206	0-4	1.78	1.409	0-5	0.01	0.09
Restlessness	0.69	1.044	0-4	0.95	1.215	0-5	0.05	0.45
Total symptom Score	4.70	3.867	0-18	8.45	6.092	0-24	0.001	0.35

[1] Kendall's rank order correlation.

The one symptom with no detectable correlation was depressive mood (correlation factor 0.09). The other five symptoms had correlation factors with a range of 0.22-0.51. The correlation coefficients for all items ranged from 0.09 to 0.66 (table 1).

The caregivers used on average 17 minutes for their ratings. Most considered the scale items relevant and stated that performing these ratings helped them to better understand the various symptoms in dementia. Only some difficulties to understand words were mentioned by some (e.g. emotional function, incontinence of faeces, distractibility and irritability).

No differences in correlation were seen if the patients had different degree of cognitive impairment. But in several items it seams that caregiver rated somewhat higher and in a few did caregiver rated lower (table 1).

Discussion

In this study we wanted to investigate whether the GBS scale could be applied to untrained caregivers in order to reliable monitor dementia patients and their symptoms. Although there were differences in staff and caregiver scores on a few items the most parts of the ratings had sufficient results. Scores on emotional and some BPSD items differed (Emotional function, Emotional lability, Irritability and Depressed mood).

It can be questioned whether the caregiver should rate these symptoms or not and caregivers shall use a limited GBS-scale? First it must be taken into account that normal caregivers have no medical education and no formal training in caring and monitoring demented persons. Secondly is most probably easier for staff members to rate symptoms of dementia objectively, they have the experiences of many patients and in this study also training in monitoring symptoms in dementia. On the other hand BPSD symptoms are usually a reason for hospitalization, much depending on how much of these symptoms the caregiver manage to cope with[17,18]. Therefore, it may be of greatest interest to have the opinion from the caregiver also with regard to these symptoms. The time spent by the caregiver to rate with the GBS scale were about 17 minutes, they clearly felt it as meaningful, and they learned about the disease. To taken account to all these aspects we suggest that the whole GBS-scale should be used by caregivers intact.

Another finding in the study is that the caregiver in some item rated higher than the personal and in other lower. Of special interest to focus on is that the difference was higher by the caregiver in the cognitive and BPSD subscales but not in ADL, and lower in the emotional impairment subscale. One reason to this can be that the caregiver has a burden, which is well documented in the literature[3,4]. Another reason can be behind this phenomenon is if the caregiver has seen the patients regular life at home and that the patient during the visit at the ward are more active and controlled more their BPSD symptoms. All these finding support that information from the caregiver is of important in the dementia process.

In addition, the results suggest that caregivers may also use the GBS scale in order to monitor clinical interventions but with caution and perhaps together with one performed by a trained healthcare personal.

The scale could also be used in the therapeutic process in caregiver support groups. The staff members who participated in the study suggested that, in connection with caregiver ratings, time should be set aside for discussion of the results. To assess functional and behavioral changes after pharmacological treatment or other health care interventions together with professionals should provide valuable feedback to the caregivers and make them feel more involved in the total care of the patient.

Finally we suggest by the results from this study that the GBS scale may provide a useful tool in the management of dementia patients and may be used by untrained caregiver with caution.

Future investigations of the GBS scale should preferably include a larger number of severely demented patients. A question is also whether training of the caregiver in the GBS-scale gives a higher correlation and if a more structural involvement of caregivers in the management of demented patients would have an impact on the clinical state of the patient, the rate of hospitalization, and the total health economic situation. Studies that address this question are clearly wanted.

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A Carer-Based Index for Identifying Early Dementia in the Elderly Living at Home

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Abstract

Data on functioning, behaviour and cognition, scored by carers, from 484 persons seen at Ullevaal Memory Clinic were related to the outcome variable „Cognitive Impairment" in subjects with a MMSE sum-score of 20 or above. Twelve of the 78 items, all scored binary, related to „Cognitive Impairment" with an accuracy of 75 % or higher. Factor analysis demonstrated only one principal construct. The six-item set that best predicted the outcome were: „Remembers shopping lists?", „Manages own possessions?", „Appears confused?", „Initiates a conversation?", „Activates him/her-self ?" and „Knows the time?". ROC-curves showed that this set was a better indicator of „Cognitive impairment" than the MMSE. A cut-off of 4/5 on the sum-score gave a diagnostic sensitivity of 0.83, a specificity of 0.81 and a likelihood ratio for a positive test of 4.4. Data from carers on activities and behaviour thus provide valid diagnostic information about an elderly person's cognitive functioning.

Keywords: Cognitive Impairment, Dementia, Elderly, Informant-based Data, Case-finding

Introduction

Dementia is a common disorder in the elderly, the prevalence in the age group 70-74 years being about 5%, 15% among those over 75 years, and more than 40% among those aged over 85[1,2]. Dementia tends to be overlooked by doctors and is frequently diagnosed at a later stage of the disease[3,4]. Many of the elderly are unaware that they are developing symptoms of dementia, or if they are aware, they try to conceal the problems. In such cases, the responsibility for initiating a diagnostic procedure will often be with a carer.

Early detection is particularly important for people suffering from cognitive problems that are potentially reversible, e.g. caused by vitamin deficiencies, depression and hypothyroidism[5]. An assessment at an early stage is a paramount for the establishment of non-pharmacological and pharmacological treatment, and there are also indications that cholinesterase inhibitors may be useful for a broader patient segment, e.g. those with vascular and mixed dementia (Alzheimer's disease with vascular components)[6,7].

A prerequisite for a diagnosis of dementia is the definite loss of cognitive skills and reduced functioning with respect to the activities of daily living (ADL)[8-12]. An informant may easily observe the presence or absence of such skills. We hypothesise that family carers, relatives, friends, home-helps, nurses and (or) unskilled health service personnel, provided with a simple check-list for activities that are typically hampered by cognitive impairment, would be able to provide data which could contribute to the detection of the elderly in need of assessment, e.g. at a memory clinic. In this study, we have wanted to establish a simple, activity-based instrument for cognitive function to be scored by relatives or other carers of the elderly suspected of developing dementia.

Materials and methods

The data came from the Memory Clinic of the Ullevaal University Hospital, which is an outpatient clinic where the patients are asked to bring an accompanying person (relative / friend / representative from the municipal health care system) who provides the informant data about the patient's daily function, behaviour and cognition. At the time when the physician performs the cognitive assessment, he / she is unaware of the information provided by the carer. The final clinical diagnosis regarding dementia is thereafter

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made by clinical consensus based upon all the available information from the cognitive and medical assessments including informant data, laboratory tests and brain imaging. The diagnostic procedure at the Ullevaal Memory clinic has been described in detail elsewhere[13,14].

For this project, we restricted the data to records from subjects with a Mini-Mental State Examination[15] (MMSE) sum-score of 20 or higher from whom data were available from a carer. According to Grut et al. MMSE cut at 19 / 20 provides a diagnostic sensitivity of 98 % for dementia in the elderly[16]. The data would therefore encompass primarily mild and suspected cases and none with severe dementia, where there is usually little diagnostic ambiguity. Dementia was diagnosed according to the DSM III-R criteria with the addition that a cognitive decline should have been present for at least six months. A diagnosis of cognitive impairment, no dementia (CIND)[17], was made if the subject had evidence of impairment in short-term and long-term memory, but otherwise did not meet the DSM III-R criteria, or if the cognitive problems had been present for less than 6 months. For the purpose of this study, we used the term „Cognitive Impairment" as our principal outcome variable, which encompassed those diagnosed with dementia or CIND.

The data to be analysed came from 484 subjects (66% women, mean age 74 years, range 43-96). Their length of education was: 7 years or less, 39%; 8-12 years, 45%; and 13 years or more, 15%. The informant was a spouse in 44%; a daughter in 22%; a son in 13%; a sibling in 5% or other carer in 16% (a more distant family-member, a friend or a representative of the municipal health care system). The patients were categorised into three diagnostic groups: 262 (54.1%) with dementia (mean MMSE = 23.5); 69 (14.3 %) with CIND (mean MMSE =27.2); and 153 (31.6 %) without cognitive impairment (mean MMSE = 28.5). Among the latter, one third were found to suffer from depression, while we detected depressive symptoms using the Montgomery & Åsberg Depression Rating Scale (MADRS)[18] in only 4% of those diagnosed with dementia.

The accompanying person provided data on ADL by the „Rapid Disability Rating Scale-2" ((RDRS-2), 18 items)[19], behaviour and mood by the „Behaviour and Mood Depression Scale" ((BMD), 33 items)[20], and general mental functioning (20 items from the „Informant Interview" of the „Cambridge examination for mental disorders of the elderly" (CAMDEX)[21]. Subjective memory complaints were scored by means of the seven questions in the memory section of the „Interview with the patients" from the CAMDEX[21].

Results

Two-by-two tables were used to study the association between cognitive impairment and all the items from the RDRS-2 -, BMD - and CAMDEX - scales; 78 in all. Many of these items are on an ordinal scale (0 - 4 or 1 - 4), and categories representing any degree of a problem whatsoever were merged, thereby creating binary items. Twelve of these 78 items that related to „Cognitive Impairment" in the 2 X 2 tables with an accuracy (proportion of subjects in diagnostic concordant cells) of 75% or higher, were pursued in further analyses (Table 1).

Table 1. Test properties of 12 items from the RDRS-2, BMD and CAMDEX with an accuracy >0.75 vs. "Cognitive Impairment"

Items	Source	Sensitivity	Specificity	Accuracy
Manages own possessions	RDRS - 2	0.84	0.70	0.81
The patient appears confused	RDRS - 2	0.82	0.68	0.79
Participates in conversation	BMD	0.81	0.61	0.76
Reads newspapers	BMD	0.77	0.67	0.75
Initiates a conversation	BMD	0.85	0.61	0.80
Activates him-/herself	BMD	0.99	0.57	0.81
Keeps track of time (day/date/month/year)	BMD	0.81	0.80	0.81
Keeps to the subject in conversations	BMD	0.80	0.63	0.76
Remembers shopping lists	CAMDEX	0.83	0.63	0.81
Does not concentrate	CAMDEX	0.92	0.53	0.87
Can think abstractly	CAMDEX	0.90	0.38	0.89
Remembers happenings	CAMDEX	0.92	0.52	0.89

Factor analysis was used to explore the dimensionality of these 12 items. Binary factor analysis should be based upon tetrachoric correlations[22] which are the Pearson correlation one would obtain if the variables were measured continuously. There were many high correlations between these 12 items, and factor analysis revealed one principal construct. A screen plot of the eigenvalues (explained variance) supports the assumption of unidimensionality among the items and shows that the first factor, which explained 68 % of the variance, dominates the other factors in this dataset. The significant drop in the contribution of the factors between the first and the rest of the factors is further evidence for the unidimensionality and allows the construction of a new index[23] (Fig. 1).

For the development of an index, in this particular case to be scored by relatives or laypersons, we decided to limit the number of items to six. Best subset multiple logistic regression analysis, using the distance measure Mallow Cp [24] was applied as a criterion to identify the six-item set that predicted the outcome variable „Cognitive Impairment" most precisely. Table 2 shows the correlations between the items of this subset. Factor analysis proved that these data were well described by one factor (Chi-square = 2.33, DF = 9, p= 0.99) that explained 71% of the total variance. We named this six-item index the „Cognition Expressed by Activities and Behaviour" - scale (CEAB-scale).

The diagnostic properties of the sum-score on the CEAB-scale (range 0-6) versus „Cognitive Impairment" was studied by means of a ROC curve and compared with the ROC-curve for the MMSE sum-score (Fig. 2). The new instrument was a somewhat better indicator of „Cognitive Impairment" than the MMSE sum-score and also superior to the informant-based instruments RDRS-2, BMD and CAMDEX (results not shown). The ROC-curve showed that a threshold value of 4/5 provided satisfactory diagnostic sensitivity (0.83) and specificity (0.81), with a likelihood ratio of a positive test of 4.4.

When checking for a health problem with low prevalence, false positives have a significant impact on expenditure and also cause unnecessary concern for those being screened and their families. Table 3 shows the percentage of false positives using the sum-score of this six-item CEAB-scale at cut-point 4/5 at various prevalence rates of cognitive impairment, e.g. at a hypothetical prevalence rate of more than 40% only every fourth case or less would be a false positive.

Discussion

Matthews et al. reported that most of the elderly with dementia in a large population-based survey were living in their own homes, and that they had been admitted to institutional care first when their disability had reached a certain level[25]. The Oslo study on the prevalence of dementia by Engedal and Haugen showed that 60 % of those with dementia lived at home[26]. The instrument presented here is intended to trace cases of suspected dementia when there is concern about the cognitive ability of an elderly person among carers, i.e. family or home-helps / district nurses. In contrast to the Informant Questionnaire on Cognitive Decline in the Elderly (IQ-CODE)[27], originally developed as an informant interview measuring change in cognition over 10 years, the present instrument can be scored without long-term knowledge of the patient. Most probably, the proportion with a verifiable impairment among persons suspected by carers to suffer from a cognitive decline is high, but we have not come across

Table 2. Correlations (tetrachoric) of the six-item set that predicted "Cognitive Impairment" best.

Items	Remembers shopping lists	Manages own possessions	Patient appears confused	Initiates a conversation	Activates him-/herself
Manages own possessions	0.60				
Patient appears confused	0.52	0.80			
Initiates a conversation	0.44	0.66	0.70		
Activates his/her-self	0.63	0.85	0.80	0.67	
Knows the time (day/date/month/year)	0.46	0.73	0.62	0.55	0.73

Table 3. Percentage of false positives on the sum-score cut at 4/5 at various prevalence rates

Prevalence	5 %	10 %	15 %	20 %	40 %	60 %
False positives	81 %	67 %	56 %	48 %	26 %	13 %

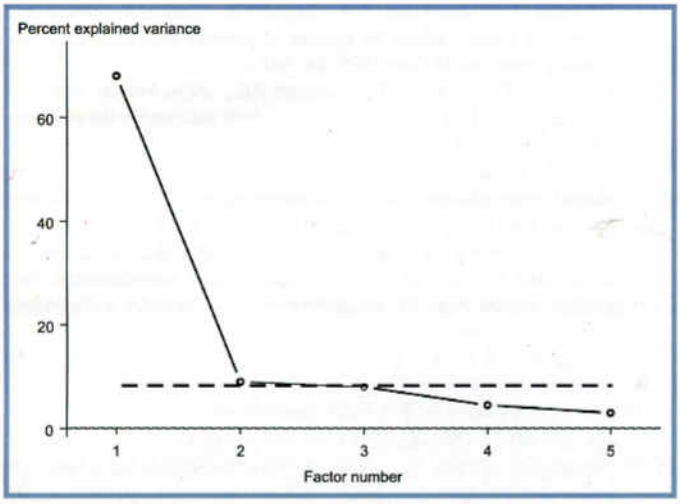

Figure 1. Scree plot of eigenvalues by construct number. Horizontal line corresponds to an eigenvalue of 1.0

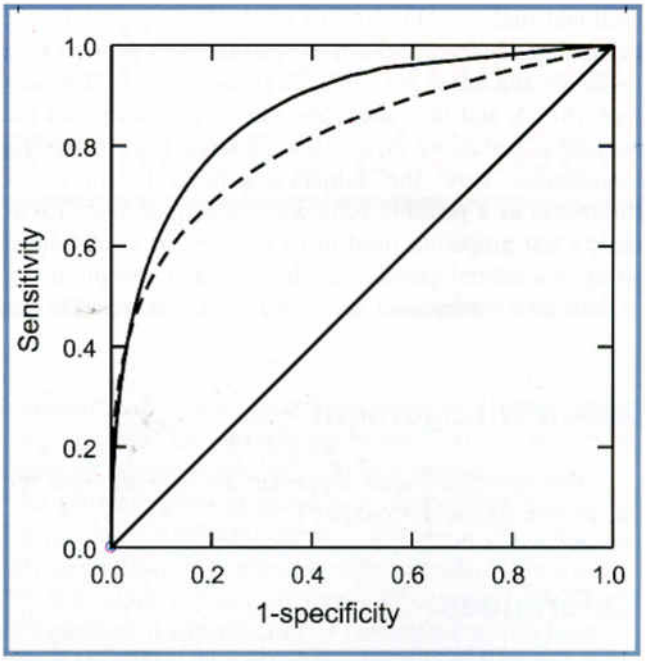

Figure 2. ROC-curves for the sum-score on the six-item set versus the MMSE sum-score (broken line)

prospective and blind studies of the validity of carers' suspicion of dementia. Therefore, the efficacy of the present CEAB-scale, e.g. the predictive values of a positive and a negative test, in the general population of the elderly cannot be estimated on the basis of the current dataset, selected from a memory clinic, where the prevalence of cognitive impairment at a level corresponding to the DSM III-R criteria for dementia was high (54%).

At the sensitivity and specificity provided by the threshold value of 4/5 on the sum-score of the CEAB-scale in this sample, the likelihood ratio (LR) for a positive test regarding diagnosed cognitive impairment was 4.4. This implies that if the pre-test probability of cognitive impairment is 0.30, the post-test probability given a positive test becomes 0.65. For this study, carried out on an actual memory clinic sample, we selected patients with a MMSE sum-score of 20 or higher, thereby excluding patients with moderate and severe dementia, where the diagnosis usually is unambiguous. If these were included, the diagnostic properties of the index presented would have appeared better, but the figures would have been unrealistic regarding the identification of subjects with mild cognitive problems, which is how early dementia cases present themselves to the family and to the clinician.

Neuropsychological test batteries have been shown to distinguish well between cases with and without dementia with high likelihood ratios (LR values of a positive test-result of 8.1 and 12.7)[28, 29]. In the well-designed study by Tierny et al.[29], the patients were followed by GPs looking for the development of dementia, whereas in the study by Incalzi et al.[28] the sample was not at all natural, since the participants were hospitalised patients. A major problem with the neuropsychological test batteries is that they require specially trained personnel, are time-consuming and give rise to a substantial proportion of drop-outs. The MMSE, which is much shorter, was shown by Grut et al.[16] to have good diagnostic properties in the Kungsholmen study of unselected subjects aged 75 years and over, and has similar diagnostic properties as our index with an LR of a positive test (LR+) of 4.3. Ritchie et al.[30] found a higher LR+ for the MMSE at a cut-off of 23/24 (8.4), but they collected their cases in institutions, where the prevalence of dementia is higher, and compared them with persons without dementia living in the community. Furthermore, the MMSE is test-based with a substantial learning effect, may offend the subject, and should only be used by skilled persons. The MMSE therefore seems unsuitable for carers to assess cognition in the elderly repeatedly.

The properties of a diagnostic test depend strongly on the population to which it is applied. Persons referred to a memory clinic may not be fully representative of the general elderly population, e.g. concerning psychiatric and physical co-morbidity. Regarding the well-documented co-existence of cognitive impairment and depressive symptoms, we suggest that the low percentage of depressive symptoms (4%) detected among our cognitively impaired subjects is biased by the possibly poor validity of the MADRS in a cognitively impaired sample[31].

Physical impairments, e.g. chronic heart failure, chronic obstructive pulmonary disease, cancer, diabetes mellitus and osteoporosis has been found to occur more frequently among those with normal mental status, who also receive more medication than the cognitively impaired, but physically unimpaired elderly[32]. By contrast, in the cross-

sectional study by Doraiswamy et al. [33], the prevalence of co-morbid medical illness in 679 Alzheimer's patients with moderate and advanced dementia (mean MMSE 11.8) was high (61%), and they conclude that co-morbidity and the cognitive status of Alzheimer patients have a strong association. How the subject's general health status influences as a possible confounder regarding the CEAB-scale's test properties need to be assessed in a subsequent study in a natural sample: unselected elderly people living in their own homes.

Acknowledgement

The work has been supported by a grant from the Norwegian Research Council.

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A Novel Mouse Model of Alzheimer's Disease

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Abstract

Objective: **To establish a novel mouse model of Alzheimer's disease.** ***Methods:*** **The NIH mice were treated with D-galactose (120 mg·kg^{-1}·day^{-1}, ip, 60 days) and $NaNO_2$ (90 mg·kg^{-1}·day^{-1}, ip, 60 days) to make the animal model of Alzheimer's disease. The Morris water-maze test, the analysis of protein content and AChE, SOD activities in brains, as well as the pathological observation of the hippocampus and cerebral cortex were carried out to evaluate the mouse model of Alzheimer's disease.** ***Results:*** **As compared with the control group, the model group showed that the escape latency time increased (P<0.01), the AChE activity in brain increased (P<0.01), the SOD activity in brain decreased (P<0.01), while the content of total proteins in brain did not show significant differences. Furthermore, the loss of vibrissae, senile plaque-like structure in cerebral cortex and the degeneration of hippocampal and cerebral cortex neurons were observed in model mice.** ***Conclusions:*** **The mouse model of Alzheimer's disease induced by D-galactose and $NaNO_2$ could simulate some characteristics of human Alzheimer's disease to some extent and would be used for study of AD and its drugs.**

Keywords: Alzheimer's disease, Learning and memory, D-galactose, Sodium nitrite, Acetylcholinesterase, Superoxide dismutase

Introduction

Alzheimer's disease is characterized by certain neuropathological lesions, including (1) the loss of cholinergic neurons in central nerve system, (2) extracellular beta-amyloid protein (Aβ) or senile plaque (SP) deposits, (3) intracellular neurofibrillary tangles (NFT) formation. There is still no effective treatment for AD, partly due to the absence of the ideal animal model of AD. So establishing an animal model that can simulate characteristics of human AD in cognitive and memory as well as neuropathological changes will promote the research of pathogenesis of AD and the screening of drugs for AD.

Previous evidence indicated that mice or rats aging model could be induced by intraperitoneal (ip) or subcutaneous (sc) injection of D-galactose, and learning and memory deficit related to anoxia could be induced by intraperitoneal injection of sodium nitrite ($NaNO_2$). Here we reported the model mice given a combination of D-galactose and $NaNO_2$. The adult mice had the same characters as aged mice and had behavioral impairments. Water maze test, analysis of related enzymes activities in brain and histopathological examinations were carried out to investigate whether this novel model is similar to the human AD.

Materials and methods

Animals and reagents

Female NIH mice, body-weight 20~24g, were supplied by the Experimental Animal Center, Sun Yat-sen University of Medical Sciences, Gaungzhou, China. Number of certificate of animal quality was No.2000A037. Total protein content detection kit, superoxide dismutase (SOD) activity detection kit and acetylcholinesterase (AchE) activity detection kit were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. D-galactose was purchased from the Shanghai Second Reagent Factory, Shanghai, China. $NaNO_2$ was purchased from the Shantou Guanghua Chemical Reagent Factory, Shantou, China.

Establishment of the mouse model of AD

Forty mice were randomly assigned to the control group and the model group. The model group mice were injected with D-galactose (120 mg·kg^{-1},ip) and $NaNO_2$ (90 mg·kg^{-1}, ip) daily for 60 days. The control group mice were injected with the same volume of saline.

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Behavioral test

The dark plastic water maze (80cm×50cm×20cm, Fig 1) was used to assay mice performance. The depth of water was 9 cm and the temperature was 24~27^{0}C. Mouse was thrown into the water with its nose towards the wall of the starting point and was trained to find the platform. The escape latency time (from the starting point to the platform) was recorded. If it was over 120s, this time was recorded. Mice were trained at the point A on the first day after the injection. From the third day to the fourth day mice were placed at the point B.

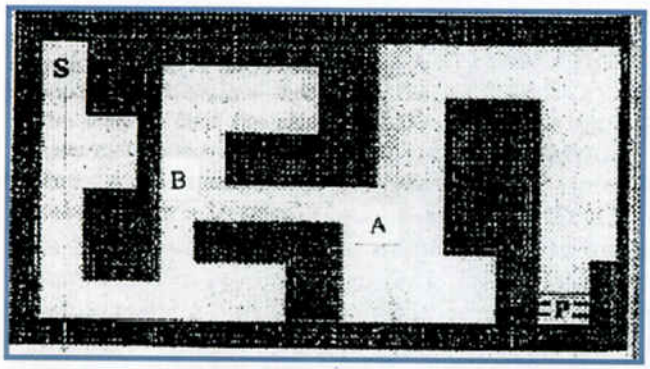

Fig 1. Mouse water maze apparatus
S: starting point P: platform

Detection of related enzymes activities in brain

After the behavioral test, ten mice of every group were sacrificed to get the brains (except for the olfactory brain). The brains were homogenized in ice-cold saline to become 10% (g·ml^{-1}) brain homogenate and was centrifuged at 3000 ×g for 10min. The supernatant was kept at ice-cold temperature for total protein content, AChE and SOD activities assays. The process was operated according to the specifications of the kits. The volume of samples was 50 μl.

Pathological examinations

The remain mice were fixed by cardiac perfusion with 4% paraformaldehyde after pentobarbital anesthesia. Brains and skins with vibrissae were fixed in 4% paraformaldehyde for 24h, paraffin-embedded section, HE and Congo Red stained.

Statistical analysis

Data were expressed as $\bar{X}\pm s$. Statistical analysis was performed with t-test with SPSS 8.0 statistical software.

Results

Results of behavioral test

Table 1. Escape latency time of mouse water maze test ($\bar{X}\pm s$)

group	number of animals	escape latency time (sec)		
		day 2	day 3	day 4
control	18	75.4 ± 29.8	51.8 ± 27.0	34.6 ± 12.6
model	17	100.4 ± 31.5*	91.9 ± 40.2**	73.4 ± 27.1**

* P<0.05 ** P<0.01 vs control

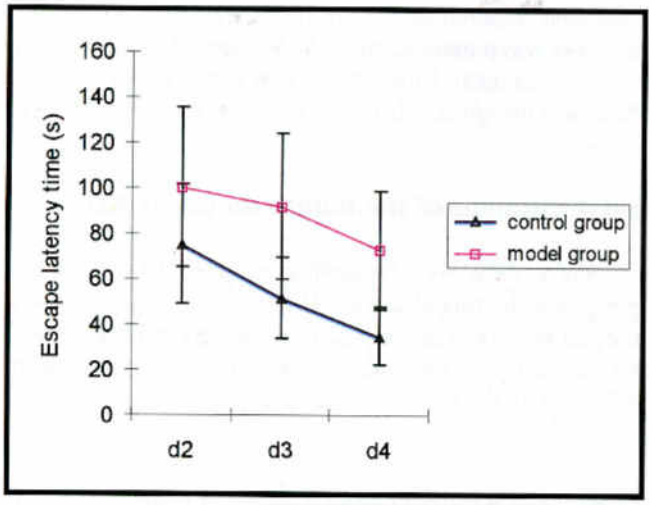

Fig 2. Escape latency time of mouse water maze test

The escape latency time of the model group was longer than that of the control group in all the time. The results indicated that the AD model mice were impaired in learning and memory.

Results of assays of related enzymes activities in brain

Table 2. The total protein content, AChE activity, SOD activity in brain ($\bar{X}\pm s$, n=10)

group	total protein content (mg·ml^{-1})	AChE activity (μmol·mg^{-1})	SOD activity (NU·mg^{-1})
control	3.33	0.735 ± 0.054	23.3 ± 2.5
model	3.19	1.029 ± 0.076**	15.9 ± 1.4**

* P<0.05 ** P<0.01 vs control

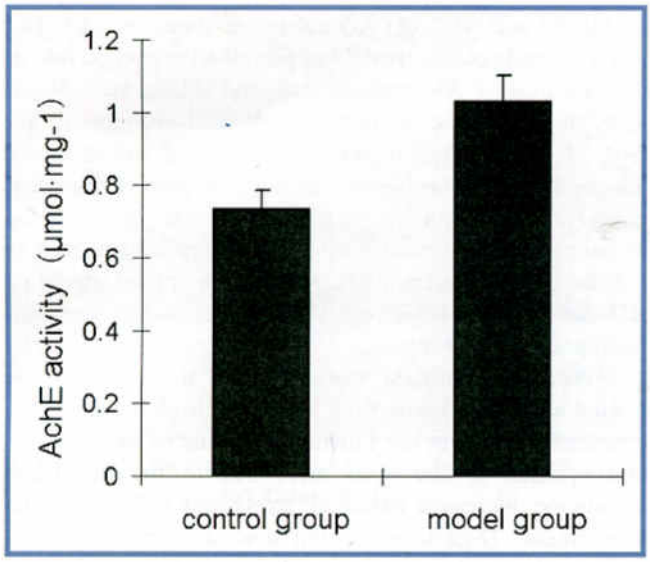

Fig 3. AChE activity in mice brain

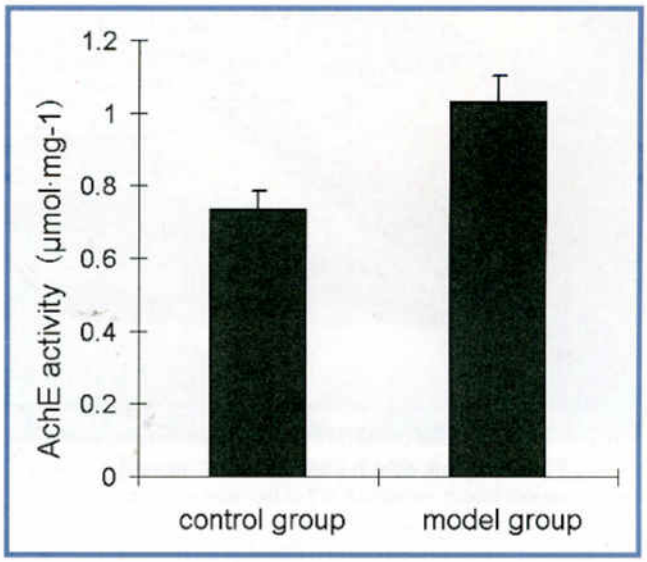

Fig 4. SOD activity in mice brain

There was no significant differences in the total protein content between the control group and the model group. But the model group had significantly increased AchE activity and reduced SOD activity.

Results of pathological examinations

The staining of hippocampus and cerebral cortex neurons of the control group was uniform and the structures of cell membrane and nucleus were integrant (Fig. 5 and 6). The dark staining degenerative neurons were seen in hippocampus and cerebral cortex of the model group. These neurons exhibited karyopyknosis and dissolved karyotheca (Fig. 7 and 8), even a broken of the pyramidal cell layer of hippocampus was observed (Fig. 9). Senile plaque-like structures could be seen occasionally (Fig.10). Maybe the behavioral abnormalities in model mice were secondary to these pathological changes.

During the experiments the general loss of vibrissae in model mice occurred at about day 40 (Fig.11). The control group showed intact hair follicles and compact hair, while the model group showed atrophic or absent hair follicles and sparse hair.

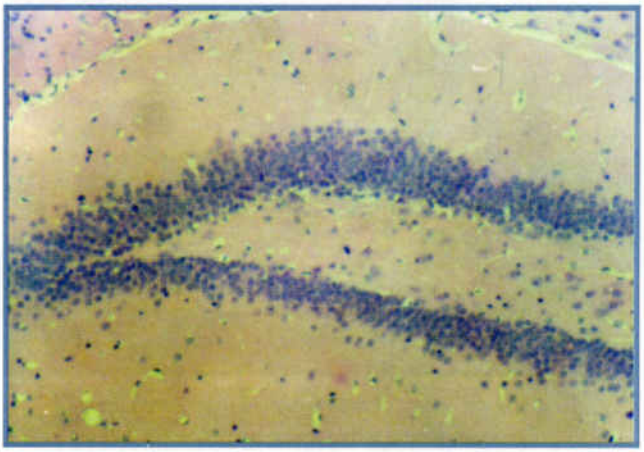

Fig. 5 Hippocampus of control mouse ×100 HE staining

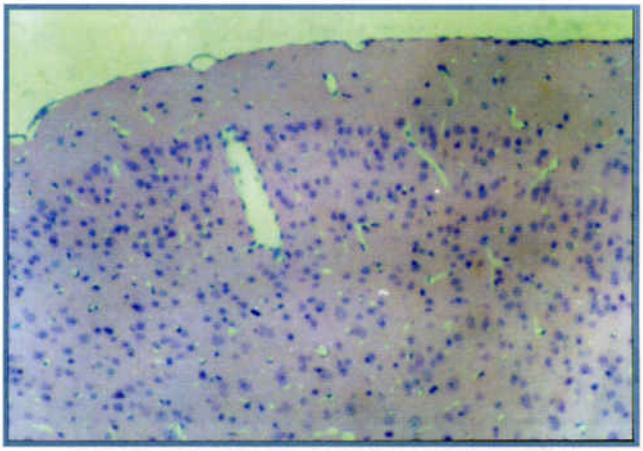

Fig. 6 Cerebral cortex of control mouse ×100 HE staining

Discussion

The main animal models of AD at present include (1) central cholinergic nerve system-impaired models, for example, the model of rat basal forebrain lesion[1] and the animal intracerebroventricular administration of neurotoxic reagents such as ibotennic acid, kainic acid, quisqualic acid, NMDA and Aβ[2]. These models could simulate the cognitive and behavior impairments, but they could not exhibit the aging character and the pathological hallmarks of AD such

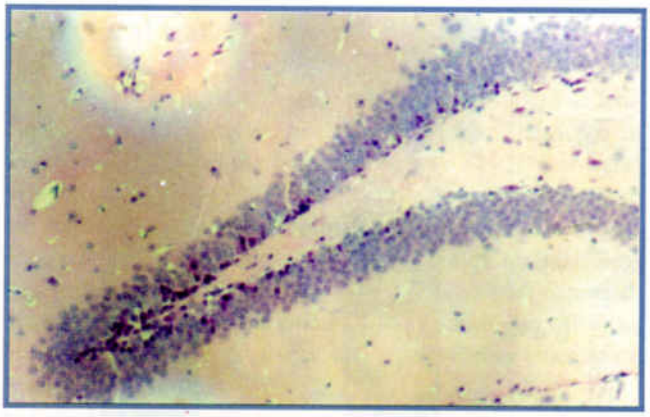

Fig. 7 Hippocampus of model mouse (denatured and necrosed neurons,dark color nuclei) ×100 HE staining

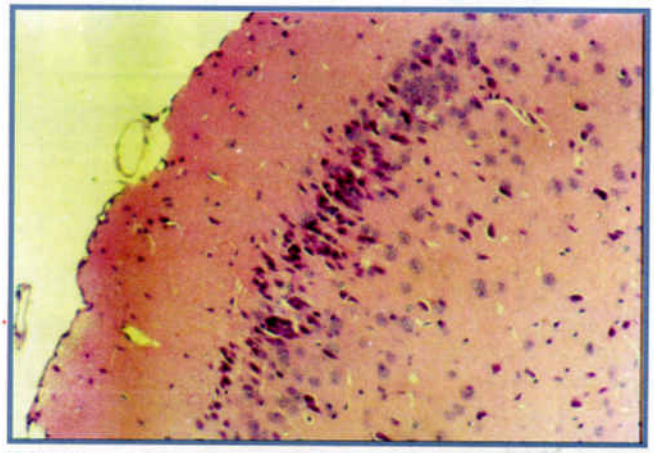

Fig. 8 Cerebral cortex of model mouse (denatured and necrosed neurons, dark color nuclei) ×100 HE staining

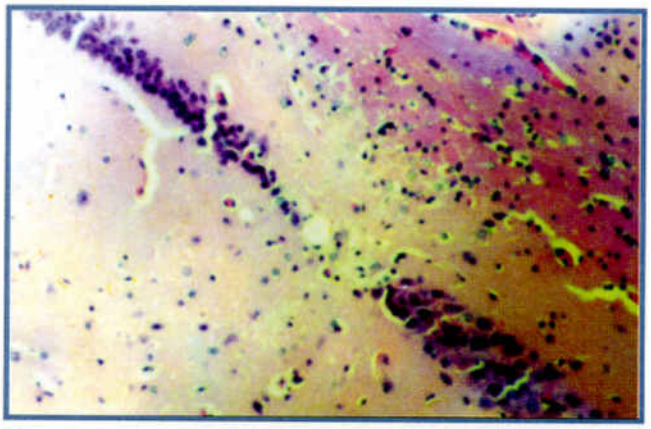

Fig. 9 Hippocampus of model mouse (pyramidal cell layer broken off) ×100 HE staining

as Aβ, SP and NFT. (2) AD transgenic mouse model. The AD transgenic mouse model has played an important role in the research of AD pathogenesis and AD-treated drugs. They displayed one or more pathological changes of AD (Aβ, SP and NFT)[3~5]. But less than 10% of AD cases are inherited[6], so the transgenic mouse model did not represent entirely the human AD. In addition, the complicated technique and costly expense limited its application. So it is a difficult but important task to build an animal model of AD that could exhibit cognitive deficits and progressive neuropathologic changes.

Previous published work reported that young mice treated with D-galactose for a long time displayed the same characters including the functional decline of body organs and systems as the aged mice due to the metabolic imbalance, increased oxidative stress and cell membrane impairment. D-galactose could also accelerate neurons degeneration and result in a reduction in the number of neurons, a decrease of SOD activity in brain, an increase of MDA and lipofuscin[7]. In our study young mice treated with D-galactose (120 $mg \cdot kg^{-1}$, ip, 60d) were used to become the mimesis of aged mice.

It was usually adopted that mouse model of memory consolidation impairment could be induced by ip of $NaNO_2$ (120 $mg \cdot kg^{-1}$). The large amount of nitrite could transform the normal hemoglobin into the methemoglobin and result in histanoxia and brain dysfunction. We adjusted the dosage to 90 $mg \cdot kg^{-1}$ for sixty days to reduce the mortality of animals. Behavioral test revealed that the escape latency time of the model group was longer than that of the control group in all the time, suggesting that the model mice exhibited a spatial learning and memory impairment in the water maze.

Acetylcholine (Ach), one of the important neurotransmitters in CNS, is synthesized by choline acetyltransferase (ChAT) and degraded by acetylcholinesterase (AchE). Most researches indicated that there was a marked decrease of ChAT activity, AchE activity and Ach synthesis in the cortical and hippocampal projection areas in patients with AD. But reports about AchE activity in animal models of AD were not consistent because of the varieties of animal models of AD. Our research revealed that AchE activity of the model mice increased, resulting in a decrease of Ach and cognitive function.

Recent evidence indicated that free radicals were involved in the pathogenesis of AD, and SOD activity was significantly lower in AD than in control brains[8]. Our results were in agreement with that of several laboratories and supported the opinion that a decrease in SOD activity was associated with aging, leading to a reduction in scavenging oxygen free radicals and an increase in neurons impairment.

The degeneration of hippocampal and cerebral cortex neurons and senile plaque-like structure in cerebral cortex in model mice were similar to the loss of neurons found in patients with AD. Furthermore, the general absence of vibrissae in model mice was observed. Vibrissae, different

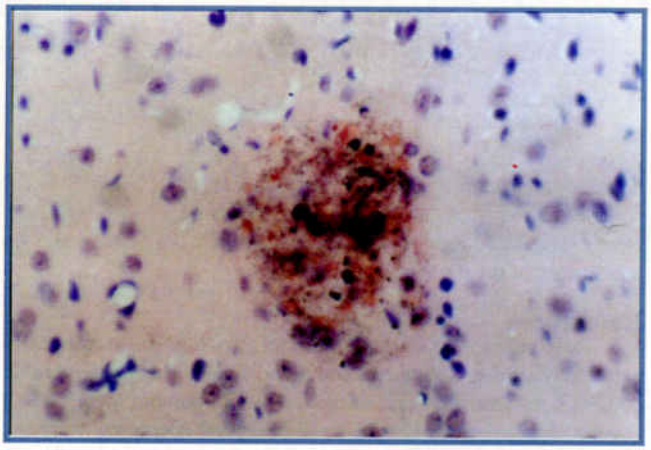

Fig. 10 Senile plaque-like structure in cerebral cortex in model mouse, × 250 Congo red staining

Fig. 11 The vibrissae was intact in control mouse (upper); the vibrissae was lost in the Alzheimer model mouse (lower).

from the ordinary fair, was very important for mouse orientation with the structure of ample nerves and blood vessels around vibrissa follicle. The absence of vibrissae in model mice implied that certain changes had occurred in their brains. It deserves further research which reagent, D-galactose, $NaNO_2$, or the combination induced this effect.

As a conclusion, the mouse model of AD induced with D-galactose and $NaNO_2$ developed the characteristics such as senescence, abnormality in learning and memory, increase in AchE activity in brain, decrease in SOD activity in brain, degeneration of hippocampal and cerebral cortex neurons, and formation of senile plaque-like structure. It appeared to be a novel mouse model of human AD. Continual research will investigate whether this mouse model could exhibit pathological hallmarks of AD (Aβ, SP and NFT).

Acknowledgments

This study was supported by grants from the Guangdong Provincial Administration of Traditional Chinese Medicine of China (98411) and State Administration of Traditional Chinese Medicine of China (D1X047B).

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