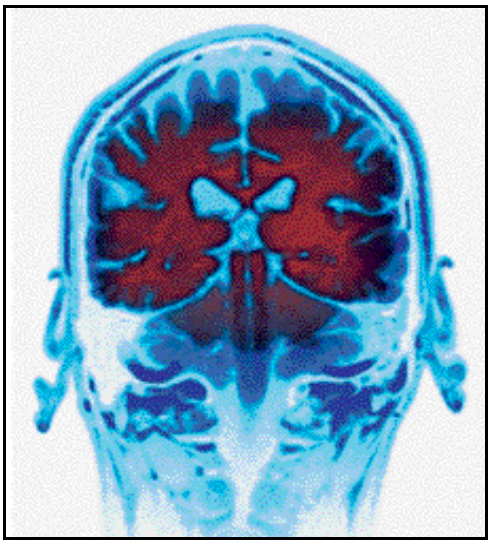

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Cell Senescence and its Consequences in Nephrology

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Senescence theories outline the importance of cumulative degradations taking place at the level of the cells that ended their division cycle, as well as the limited replication ability of the cells with mitotic potential. The senescence fenotype is assessed to reflect the carrying on of a settled genetic programme, but cumulative cell degradations play a special part, as well as the limited capacity of reparation of these cells, external factors being very important. The lowering of cell number reflects rather the decreasing of repairing capacity. In vitro, some cell types loose their ability of suffering the apoptosis process, gaining some abnormal characteristics and the accumulation of such cells is suggested to contribute to senescence and to the emergence of the diseases determined by this.

Senescence biomarkers can be viewed in vivo, being represented by lipofuscin accumulations, advanced products of glycosylation and β-galactozidase associated to senescence.

The proposed hypotheses for explaining the aging of the cell and organs is based either on the existence of in or out the cell lesions, or on the programmed alterations of genetic expression.

The functioning of the tissues and the senescent fenotype reflect:

- The accumulation of cell degradation and/or senescent cells;
- Cellular loses;
- The loss of the ability of answering through replication, as a result of stem cell senescence;
- Modifications of extracellular matrix;
- Perturbation of inflammatory, imunologic processes and of those of endocrine control.

Age can determine modifications of gene expression at the level of the cells with mitotic potential, while successive lesions play an important role in the senescence of the cell that no longer divides.

It was proved that once the cell ages, there is a shortening of the telomeres, this process being extremely intensive at the level of the cells of kidney cortical. The significance of loosing the telomeres is not clearly established, but the appearance of a heterogenity at the level of kidney cells, associated with loosing the nuclei, could play a part in cell senescence.

The involvement of oxidative stress in senescence is sustained by the fact that the modifications associated with senescence are the consequence of oxidative degradation of nucleic acids, cell membranes and proteins.

Lipofuscin (LF), a yellow-brown pigment, accumulates in the cells that no longer divides, being localized in small granules from the secondary lizozoms. LF is made up from lipidic and proteic residues, as a result of lipidic peroxidation. It was proved that antioxidant substances administration inhibits LF formation.

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Oxidative modifications of extracellular proteins finally lead to the emergence of final glycozylation products. These products, that are increased at elderly, are also involved in the emergence of specific modifications of senile kidney.

The activity of β-galactozidaze associated with senescence is considered a marker of the aging processes, being identified at the level of retina, liver cells and, more recently at the level of kidney cells.

The normal process of aging is much more accelerated in case of additional factors, external ones (high blood pressure, heart failure, and so on).

Knowing the mechanism of senescence is of special importance for human pathology, for future prophylactic measures. Probably, future genic or drug therapies will be able to delay the mechanism of cell senescence, so the life of kidney cells will be prolonged, these cells being able to cope successfully with the injuries caused by different kidney disease or other disease.

Non-Pharmacological Interventions for Patients with Dementia and their Caregivers

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Abstract

Recent years have seen the growth of research interest in pharmacological but also in non-pharmacological interventions in dementia. Our discussion is about cognitive, functional and behavioral side of rehabilitation of patients with dementia as well the educational interventions to caregivers. All these approaches have the advantage being safe and effective with demonstrated psycho-educational and cognitive-behavioral benefits, as well being practical and flexible. Interventions to improve cognitive performance: reactivating occupational rehabilitation (memory training, manual/ creative activities, improving sensorimotor functions and self management therapy), validation therapy, programs of facilitating personal expression, reminiscence, Montessori-inspired methods, exercise therapy, dancing therapy. Interventions to improve functional performance: studies show that behavior modification, scheduled toileting and prompt voiding can reduce urinary incontinence. Also graded assistance, skills practice and positive reinforcement can increase functional independence in persons with dementia. Interventions to improve problem behaviors. Music particularly during meals and bathing, walking or other forms of exercise, light exercise, Simulated presence therapy, massage, comprehensive psychosocial care programs, pet therapy, commands issued at the patient's comprehension level, rright light, white noise, cognitive remediation. Interventions for caregivers: comprehensive, psychoeducational caregiver training, support groups, computer networks to provide education and support to caregivers, telephone support programs. Care environment alterations: special care units within long-term care facilities, homelike physical setting with small groups of patients, short term, planned hospitalization of 1-3 weeks, Provision of exterior space and changes in the bathing environment. Finally, more evidence-based studies must explore the benefits of various interventions for patients with dementia and their environment. However the best intervention to improve cognition, behavior, activities of daily living of the patients with dementia and the mental health of caregivers is a combination of cognitive therapy, medical treatment and caregiver's counseling.

Introduction

In recent years, there has been significant progress in understanding the pathogenesis and pathophysiology of AD and this has led to the development of new therapeutic strategies and introduction of several pharmacological agents and non pharmacological interventions targeting cognitive and neuropsychiatric symptoms.

It is well known that cerebral plasticity at young ages is highly dependent upon the quantity and quality of the stimuli of the environment. Studies conducted on second language acquisition have been shown to lead to cortical reorganization. Some years before we could not imagine that we could expect to improve brain plasticity at higher ages. Recent studies showed that the dramatic disconfirmation of a long-held, central assumption about the brain – that new cell bodies cannot emerge in adulthood – is not true. In both humans and animals recent data shows that in the hippocampus, new cells can indeed be produced[1,2,3]. This finding follows close on the heels of another revolutionary discovery of the last decade – the demonstration that the adult brain can show large experience – dependent change in neural circuits, including dendritic and axonal sprouting[4].

We have to know that the use of behavioral therapy and behavioral approaches involving the application of learning principles have been demonstrated to be effective as an intervention technique with the

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elderly for a variety of problems in a variety of settings. For example intensive cognitive remediation program on executive processing in patients with schizophrenia[5] and interventions for confusion and dementia such as reality orientation[6].

Some PRINCIPLES are described below: (1). when using behavioral approaches it is important to remember that the behavior of organisms is maintained by reinforcement. If a person exhibits a behavior, it is because the behavior is meeting a need. (2). For the behavior to be changed, either the need has to be satisfied by a different behavior or the behavior has to have a different consequence. (3). Behavioral approaches are extremely powerful in maintaining and changing behavior and often occur coincidentally. (4). To change behavior purposefully, it is important to keep in mind that the meeting of needs by a different specific behavior or the reinforcement of a new behavior needs to be consistent for the entire daily environment. (5). Non-pharmacological interventions are usually first-line in dealing with milder behavioral and psychological symptoms of dementia (BPSD) although there is limited research supporting the use of many interventions. (6). Despite the fact that research supports the effectiveness of behavioral interventions for specific behavioral problems, these strategies have not seen widespread use in nursing home settings. (7). Another reason for the limited use of behavioral approaches may be that nursing home professionals are not aware of the effectiveness of such interventions. (8). Other reasons for the limited use of behavioral approaches in long term care settings may be that implementation requires not only caregiver training, but also consistent maintenance of appropriate environmental contingencies. (9). For moderate to severe BPSD, medication is clearly indicated, often (though not always) in conjunction with non-pharmacological interventions. However the decision for medication has to be taken carefully and any treatments should be monitored and adjusted accordingly. (10). Patients with dementia may become fearful, anxious or restless because they cannot properly see or hear their environment. Thus, patients need to be examined routinely to evaluate vision and hearing. (11). Environmental interventions are also very helpful. The ideal environment for a patient is one that is non-stressful, constant and familiar. (12). A general approach includes: (a) identifying the target of BPSD, (b) gathering information on BPSD, (c) identification of the triggers the triggers or consequential events of specific symptom, (d) setting realistic goals and making plans, (e) encouraging caregivers to reward themselves and others for achieving goals, (f) continually evaluating and modifying plans

A. Interventions to improve cognitive performance

There are different procedures for cognitive rehabilitation in early stages, in mild and mild to moderate stages than in moderate to severe stages. These interventions could be useful for all the domains of dementia: cognition, activities of daily living, behavior and caregiver's burden. The most frequent all over the world are:

1. Early stage memory loss support groups

These programs demonstrate the variety of support programs for people in the early stages of AD and families around the world. They highlight the uniqueness of each program including services, research, education and training and advocacy and motivate participants without early stage programs to begin them.

Although there has been a critical gap in services for individuals diagnosed with early stage programs are now providing follow up to diagnosis and a supplement to pharmacological treatments. These programs have the added benefit of reaching families in the early stages and familiarizing them with all AD services sooner. For example, Chicago matches early stage individuals with medical students in a geriatric education program. New York's early stage group members participate in professional training and media events, and also have a writing group. Australia has evaluated the benefits/outcomes of early stage support groups and also has a national network for facilitators. Canada has an early stage program with a social/activity focus, in addition to support/discussion groups. Cleveland offers time-limited education sessions, while others offer ongoing, open-ended groups.

The purpose of these early stage support programs is: (1). To support the educational, social, emotional and coping needs of the person (and his/her family caregiver) in the early stage of dementia/memory loss (2). To provide a safe and positive setting for the person to express feelings about losses and learn to build on his/her strengths and (3). To maintain the self-esteem of the person by including him/her in planning for future needs.

We also in Greece have programs for early stages of Alzheimer's disease that show that patients can maintain their cognitive functions more if they attend these programs

We could suggest an index of managing memory loss: (1). Being open and honest about my memory loss with people that I meet. (2). Accepting my memory loss and finding ways to overcome it. (3). Establishing a regular routine and sticking to it. (4). Taking over my memory loss with someone I trust. (5). Keeping my fears and feelings secret.

2. Methods of facilitating personal expression

Although it has been alleged that individuals with AD experience a

loss of self as a result of the disease, there is good reason to challenge this view. Using the framework of Social Constructionist Theory[9], it becomes evident that AD sufferers, even in moderate to severe stages of the disease: (a) can and do experience and express selfhood in a variety of ways, verbal and no-verbal communication, (b) are vulnerable to losses in some aspects of selfhood, but those losses are not necessarily related to the disease, rather to the ways in which health others approach and interact with the person with AD, (c) can be helped in a variety of ways to avoid such losses of selfhood as might occur. Sabat[10] illustrates the potential for the acknowledgement, expression and validation of the three types of selfhood expression in persons with AD: (a). There is a self of personal identify that remains intact even into the moderate to severe stages of the disease; (b). There is a self that is comprised of mental and physical attributes, which can also persist far into the course of the disease; (c). There are other aspects of the person, the socially presented persona, which can be lost – via: (1). Their spoken words and social interactions in support groups; (2). Their written words in a writing workshop – when participants of this group write, they are remembering; (3). Their expressions in visual forms via the use of art therapy.

Reactivating occupational rehabilitation (memory training, manual/ creative activities, improving sensorimotor functions and self management therapy) proved more efficient in improving cognitive performance, psychosocial functioning, emotional balance and subjective well-being than functional rehabilitation (functional occupational therapy, physiotherapy and speech therapy)[11].

3. Reminiscence

There is a program in Europe "The age exchange training program" which is internationally known for work in all fields of reminiscence, including theatre, publishing exhibitions, cross-generational projects and training workshops. Several scholars noticed the value of reminiscence before recent groups who working in both Europe and USA. One of the first was Socrates who stated: "The unexamined life is not worth living".

Another was Tolstoy who described the difficult death of Ivan Illych, a mediocre bureaucrat who was suffering with cancer and knew death was approaching. Ivan was despairing and rude to his family who tried to comfort him. He turned his head to the wall and after a time began to "pass his life in review". For three days the family heard him moaning and groaning and then there was silence. When they entered the room, Ivan was at peace, kissed them, closed his eyes and died.

Skilled reminiscence work helps to generate stimulating and enjoyable creative activities. It enables people to make rewarding social contacts with people of different ages and ethnic backgrounds and it enriches personal and professional work satisfaction for a wide range of cares, teachers and artists. One can talk about reminiscence from many different points of view: reminiscence as a tool of communication between carergiver and caree, reminiscence as a way of reinforcing the identity of the demented person as well as his or her carer and reminiscence as giving a sense of coherence to the carer and helping him or her to cope with the caring situation.

In a study reminiscence program decreased depression for the carer and improved the cares opinion of the caree's mood and behavior. The carer's function was improved in two areas, socialization and walking.

Our reminiscence program is perhaps the most favorite intervention program in Thessaloniki. The other most favorite interventions in our city is the approaches for memory, orientation and executive function. An example of intervention for disorientation and memory loss is given by Hunley and Lusty, 1984. They reported the case of an 84-year-old female AD patient who was having difficulty remembering items of her personal information and her daily schedule. She was given a diary containing her schedule and important personal information (brother's name etc.) and was taught to look in the diary when she couldn't remember these things. After 2-4 weeks the patient was able to consult her diary.

4. Reality orientation

It is an exercise program of orientation in time and space introduced by Hanley IG in 1986.

5. Physical exercise[12]

The first study investigated a walking program specifically designed for chronically confused residents who had a history of disrupting wandering behavior[13].

We have finished an exercise program with patients with Alzheimer's disease which was sponsored by European Commission which showed that patients with dementia can be helped very much by these programs in cognitive, functional and behavior symptoms and their life cold be changed[14].

The most striking thing about patients with dementia of Alzheimer type is their ability to walk until the late stage. Sometimes their ability to keep walking could be considered as agitation for which a drug could be prescribed. But they feel that their mobility is their strength. We can use it to help them. It is something that they can do independently and is a way to interact with the world. It certainly is not causing them or others any apparent harm.

6. Validation therapy[15,16]

Validation therapy is now an integral part of our repertoire of communication and group therapy techniques

for those suffering from dementia. It focuses on the emotional, rather than factual, content of what people say. It assumes that the behavior and speech of the disorientated person has an underlying meaning, and that disorientated elderly people return to the past in an attempt to resolve unfinished conflicts by expressing feelings previously hidden and to relive memories.

The validation approach means accepting the feelings of the demented old person; to acknowledge their reminiscences, losses and the human needs that underlie their behaviors without trying to insert or force new insights. Validating includes: reflecting a person's feelings, helping them to express unmet human needs, restoring well-established social roles (which in turn help to motivate expression of social behaviors) facilitating feelings of wellbeing and stimulating interaction with others[17].

History of Validation Therapy: Naomi Feil, an American Social Worker, developed VT while working as a group therapist in the Montefiore Home for the Aged, Cleveland, Ohio. She recognized the limitations of reality orientation, which when used with very disoriented people produced distress and no discernible benefit. This disillusionment led her to develop an alternative interactive technique, in which the goal of achieving a grasp of reality is superseded by that of communicating with disoriented people in whatever reality they are in, with the result of easing distress and restoring self-worth.

In 1987, Christine Bleathman and Ian Morton received funding to conduct a pilot study to assess the effectiveness of validation therapy (VT) an interactive technique and group therapy for people with dementia. In 1988, the authors published a brief introductory article about VT. The response to the article was enormous. However, what was apparent, then and now, is that it was not just VT which produced this level of interest but the thirst for a variety of different interventions to use with people suffering from dementia. Cares, both paid and family, are keen to add to their repertoire of interactive skills in order to deal with memory difficulties, dysphasia and disorientation, all of which make communication difficult.

It recognizes the emotional needs of dementia sufferers and highlights inadequacies in the attempts to orientate the disorientated, a technique which gave little recognition to the experience of dementia - an experience characterized by feelings of loss, fear and isolation.

Examples:

1). It provides a therapeutic technique for responding to sufferers who refer to their parents in the present tense as if they were still alive. Dementia sufferers talk of going home to parents, express concern about them, use parental authority as a reason for doing or not doing something, or ask when their mother or father will visit. It has been suggested that these perpetual requests for parents should be interpreted as a cry of distress or a need for security, rather than a sound from the faded past and that can be explained in terms of Bowlby's Attachment Theory. By acknowledging the emotions that the experience of dementia creates, VT provides a therapeutic framework in which the multiple losses experienced by sufferers are acknowledged.

(2). VT also provides carers with techniques to communicate with people whose speech is confused and/or dysphasic. Two word statements of emotion, e.g. "you are frustrated" or "you are sad" allow the sufferer to have his/her feelings acknowledged. It has been suggested that these statements should be expressed in a tone appropriate to the emotion. Rephrasing what the sufferer has said using vague pronouns and key words, e.g. "They left you alone did they?" demonstrates that you have heard what has been said. Other techniques described include mirroring non verbal behavior, identifying the sufferer's preferred sense (visual, auditory, or kinaesthetic) and linking behavior to the unmet need. The techniques have to: (a) centering of caregiver, (b) building trust, (c) rephrasing (you mean you don't want to live any more?), (d) asking the extreme (what was the worst), (e) imaging the opposite, (f) reminiscing, (g) maintaining close genuine eye contact, (h) using ambiguity, (i) using a clear low loving tone of voice, (j) mirroring, (k) linking the behavior with an unmet human need, (l) touch, (m) music.

Feil advises asking "who", "what", "where", "when", "how", but never "why" in response to confused and/or dysphasic speech (Feil, 1993).

There are some principles of validation therapy: (1). Feelings when validated, lose strength, (2). When recent memory fades, early memories return, (3). humans have many levels of awareness and (4). Empathy builds trust, restores dignity.

The benefits of the validation therapy are (1). Residents display more social controls, (2). Increased verbal and non-verbal communication, (3). Decreased crying, pacing, pounding, (4). Co-workers experience less frustration/burnout, (5). Decrease in co-worker turnover rate (6). Families experience less frustration.

7. Montessori-inspired methods are promising

Maria Montessori was the first woman M.D. in Italy and worked with children of lower economic status in the early part of the 20th century in Rome. She thought that education would give these children a better quality of life and designed educational activities for children based on the abilities they had available. The Montessori Method is a method of creating and presenting activities based on models of learning, memory and rehabilitation. Materials usually are taken from the everyday environment which leads to a sense

of familiarity with the materials. Each activity is presented at it simplest level and each activity that follows builds upon the previous activity. The philosophy of the Montessori method is to create persons who are as independent as possible, able to make choices, and who are treated with respect and dignity. Many Montessori-based activities are based on skills that are learned when people are young. Because these skills often remain intact into the later stages of dementia, activities constructed on them can be performed successfully. The Montessori method provides environmental support, repetition, feedback and closure, all of which are extremely important for the older adult with dementia. Each activity is broken down into individual steps. Using the Montessori Method lets you choose activities that are appropriate for different stages/levels of cognitive abilities in dementia. In addition, specific activities can be modified for use with different individuals with dementia[19].

B. Interventions to improve functional performance

1. Improving nutrition[20]

There are some principles that could help us to improve nutrition: (a). We have to know that when patients have altered perceptions they may stop eating because of paranoid and delusional ideation about food and fluids. Then treatment of the underlying psychotic process will help. (b). The individual with dementia can eat independently for as long as possible.

Caregivers have to take care of the environment at mealtime, the meal itself, the caregiver's approach at mealtime, the assessment of cognitive, physical abilities, the patient's ability to eat independently, the appetite of a person with dementia and emotional status of the patient. Perhaps it is necessary for brief step by step instructions, for example: "Margaret, here are some delicious scrambled eggs with cheese", "Open your mouth", “Chew, Margaret". For someone who is more impaired, you may also need to lightly touch the chin as a reminder to chew food in the mouth: "Close your lips", "Swallow".

Music (of the patient's preference)[21] reduced agitation, aggression and mood disturbance under various conditions including eating and bathing[22,23].

2. Therapeutic gardens

Until recently care settings for those with Alzheimer's disease have not focused on the outside environment and the need to encourage older adults to spent time outside in fresh air and sunshine. Carefully planned environmental settings, whether interior or outside spaces, help to reduce challenging behaviors such as agitation, incontinence and wandering; poorly designed environments , conversely, can precipitate agitation and contribute to disorientation, confusion[24].

3. Pet therapy

There are no many studies which could show the beneficial results of pet therapy. However in every day clinical praxis we hear from caregivers that patients with dementia take care of their animals and they play and communicate with them.

4. Self care interventions

Self-care skills begin to diminish in the middle stage of AD and are usually absent in the later stages. Behavioral gentologists apply skill training and reinforcement techniques to help elderly patients reacquire self-care skills. Some years ago in 1978 Mishara reported patients who were treated in a token economy setting in which they earned tokens for completing their own self care skills, engaging in social activities and so forth.

Burgio et al in 1988 studied four cognitively impaired elders who were incontinent. They initiated a treatment procedure of prompting the patient to use the toilet every 2hrs. All were capable of independent use of the toilet before treatment but seldom did so. The authors found that prompting toilet use resulted in significant decreases in the number of incontinent episodes.

C. Interventions to improve problem behaviors

Assessing behavior changes in Alzheimer's disease

It is necessary to carry out a physical examination for exclusion of a physical illness and a full psychiatric evaluation. Specific behavioral instruments BEHAVE-AD (Reisberg, 1987), Dementia Symptom Scale (Loveck, 1994). California Dementia Behavior Questionnaire (Victoroff, 1997), Cohen-Mansfield Agitation Inventory (CMA I– Cohen-Mansfied, 1996), the Consortium to establish a registry for AD (CERAD) Behavioral Rating Scale for Dementia, (Tariot, 1995), RMBPC – Revised Memory and Behavioral Problems Checklist, ABID – the Agitated Behavior In Dementia, NPI – Neuropsychiatric Inventory), Columbia University Scale for Psychopathology in Alzheimer's Disease (CUSPAD, Devanand, 1992) and the Cornell Scale for Depression in Dementia (Alexopoulos, 1988) are preferable to the more general CGIC for detecting and quantifying behavioral disturbances in AD patients.

Gender differences were found in assessment of BPSD. 28,367 residents with AD were examined. Men were more likely than women to exhibit behavioral problems such as wandering, abusiveness and social impropriate (59% versus 50% for any behavior problem). Hallucinations and delusions as well as depression were equally prevalent

in men and women. Nevertheless, men were more likely to receive psychoactive medications[25].

There are some principles in non-pharmacological interventions that include: (1). Identifying the target of BPSD, (2). Gathering information on the BPSD, (3). Indentifying the triggers or consequential events of a soecific symptom, (4). Setting realistic goals and making plans, (5). Encouraging caregivers to reward themselves and others for achieving goals, and (6). Continually evaluating and modifying plans.

1. Art therapy

Historically, the arts and humanities were seldom recognized for their capacities to advance health care and promote quality of life within the fields traditionally reserved for the "hard sciences". However, in recent years, we have seen an increased awareness of the potential value of music, art and other recreational therapies in persons with dementia.

These interventions are usually first-line in dealing also with milder behavioral and psychological symptoms of dementia, although there is limited research supporting the use of all these interventions. The Behavioral and Psychological symptoms that are most responsive to non-pharmacological interventions include: mild depression/apathy, wandering/pacing, repetitive questioning/mannerisms.

Usually we have to begin by modulating the environment. Then mediate those factors that aggravate the behavior, a noisy roommate or excessively bright lighting, for example. Strategies also should optimize social and physical stimulation using techniques reported above such as validation, support and reminiscence. All these techniques could help maximize functioning and independence, enhance communication, promote a sense of security and predictability, modulate behavior and help enhance mood.

The therapeutic process also should be directed at caregivers. Physicians should be encouraged to educate caregivers and patients about diagnosis, the prognosis and non pharmacological strategies that are likely to prove most useful, recognizing that caregivers have a critical role to play in patient management.

2. Music therapy[26]

Great strides have been made in the study of music in persons with dementia. Research has focused on two basic categories of music: a. classical/relaxation for groups of patients; and b. individualized or preferred music as one on one intervention. Two primary studies have led to the branching and development of research in these areas.

Gerdner[27] provided individualized music via audio cassette to five agitat nursing home residents using a pre-post design. Sessions lasted 30 minutes, and data were collected before, during and for 1 hour after the sessions. Results showed a significant reduction in agitation from the presession period compared to 1 hour after the session.

Goddaer and Abraham[28] were the first to systematically evaluate the effects of relaxation music in patients with dementia. They reported a significant reduction in the overall level of agitated behaviors (63.4%) as well physically nonaggressive behaviors (56.3%) and verbally agitated behaviors (74.5%) in the 29 patients studied. These results were supported when the study was replicated by Denny[29]. Other researchers have also investigated the effects of classical/relaxation music in patients with dementia – there are at least 78 references.

Psychosocial interventions for treating BPSD

There are interventions for behavior excess that includes behaviors such as disruptive vocalization (DV) and wandering and interventions for behavior deficits, such as inactivity and non-social behaviors for example depression. There are some principles that could help: (1). An optimal, tailored and flexible care package which provides support at the times of greatest need can often preclude the need for additional treatments. (2). There are almost always factors which can trigger or reinforce problems. A detailed assessment can usually provide some helpful indications of things that can be changed to reduce the problem. (3). Structured interventions such as a short, planned walk, personalized music, structured social interaction built on reminiscence and appropriate activities may all be helpful in reducing behavioural problems. There is a clearly a place for drugs in treating behavioral problems but the decision has to be taken carefully and the following points considered: (1). The person should be assessed before drugs are prescribed to rule out a physical cause for their behavior such as infection, constipation, pain and sight or hearing difficulties. (2). Many less severe problems spontaneously resolve themselves over three or four weeks with appropriate monitoring. (3). Changes in the person's environment or a psychological approach may be a more effective treatment. As far as the interventions for behavioral excess we could describe (A). The long-term-care settings and (B). Community-based settings.

A. Long-term-care settings

a. Emotion orientated approaches: These interventions, which include validation and sensory integration, are thought to improve emotional and social functioning by supporting patients in the process of coping with the cognitive, emotional, and social consequences of dementia. These interventions attempt to capitalize on the subjective experiences of patients. Toseland et al[30] compared a validation group to a social contact group and a control group that received usual care.

After 1 year, patients in the validation group were physically less aggressive than patients in either the social contact or usual treatment groups. However, behavior such as repetitive movements, pacing and hiding objects decreased less than in the social contact and control groups. Holtkamp et al and Spaull and Leach examined the effects of sensory integration on various dementia-related symptoms. Sensory integration involves activities in which various sensory perceptions and experiences are stimulated. The aim is to create an atmosphere of trust and relaxation. During the first study, the sensory integration session, patients displayed fewer repetitive behaviors and less restlessness. In the second study the authors reported a decrease in "problematic and provocative" behaviors during the sessions and up to 10 minutes after the sessions.

b. Stimulation-oriented approaches: Lawton's[33] Press-Competence Model posts that dementia patients perform optimally when the environmental press (i.e., level of stimulation) matches the patient's ability to adapt to the demands of the environment (i.e., competence). Too much or too little stimulation can result in BPSD. Burgio et al[34] investigated the use of environmental white noise as a treatment for DV. Environmental white noise consisted of "gentle ocean" and "mountain stream" audiotapes played for the residents through headphones. Results of this small sample study (n=9) showed that residents displayed 23% fewer DVs in the nursing unit when they were listening to audiotapes, compared with a no-audiotape phase. Finally some recent studies explored the effects of interventions that included multiple and varied activities in combination: sight, music, touch, smells, taste and physical and social activities. Although findings were mixed, each study reported significant reduction in agitation.

Examples of stimulating communication through sensory activities: Sight: items that are different colours, from bright to dark, for example a lava lamp, blown bubbles, shiny or metallic wrapping paper. Sound: different music and sounds such as the person's favourite music, relaxation tapes, wind chimes. Or you could try stillness and quiet. Smell: stimulating or relaxing aromas, for example, flowers, herbs, candles, polish, soap, grass or food. Touch: tactile objects or your personal contact, for example, different fabrics, items with soft or rough surfaces, warm and cool items. Taste: different food and drinks such as sweet or savoury foods, iced lemonade, tea or coffee.

An example of the benefits of sensory activity is the case of a daughter who wanted to care for her mother at home but was finding it difficult to cope because her mother constantly followed her around. During the day mother and daughter could enjoy activities together but by the evening, when the daughter's husband came home, she was tiered and wanted to spend time with her partner. Her mother went to one specialist unit for a complete assessment of her needs. The multisensory assessment, carried out in the sensory room indicated that she enjoyed tactile stimulation. Using this information the staff member introduced her to exploring bead boxes, soft toys and arranging ornaments and, using this information, her daughter prepared a range of activities for her mother to do in the evening after their meal. These activities allowed the daughter and her husband more time together in the evening and enabled her to continue looking after her mother at home, which is what she wanted to do.

c. Behavior therapy and communication training: Behavior therapy techniques involve a careful description of the target behavior along with its current antecedents and consequences. Environmental antecedents can elicit a behavior problem, and consequences, such as staff attention can serve to maintain the problem over time. Behavior therapy involves changing the antecedents and/ or consequences of the target behavior with the intent of changing its frequency and/or duration. Preliminary results that nursing assistants offered more verbal prompts and activity announcements to residents after in-service and on-the-job training35. Communication skills were also improved, as reflected by increased use of 1-step instructions and of a 5-second delay between verbal prompts and provision of physical assistance.

B. Community-based settings

A number of excellent literature reviews are available on intervention strategies with unpaid caregivers. Their results can be distilled to the following: (a) multi-component interventions that blanket caregivers with an abundance of services, supports and therapies tend to generate larger effects than narrowly focused interventions. (b) single-component interventions with higher intensity (frequency and duration) also have a greater positive impact on the caregiver than do lower-intensity interventions and (c) the most recent group of intervention studies suggest that important synergies may be achieved by simultaneously treating the patient and altering the social and physical environment of the caregiver-patient dyad (e.g.), pharmacotherapy for the patient and skills training for the caregiver.

REACH is a 5-year cooperative agreement funded in 1995 by NIA and NNR[36]. REACH grew out of a body of knowledge that recognized well-documented burdens associated with family caregiving and out of the emergence of a number of promising interventions for reducing caregiver burden.

The types of interventions include: (a) individual information and support strategies, (b) group support and family sytems therapy, (c) psychoeducational and skill based training, (d) home-based environmental interventions and (e) enhanced technology systems.

Examples of interventions

1. Wandering: A confused person will often forget that he or she is supposed to be at a particular place and requires frequent reassurance about where they are and why they are there (Hussian, 1982). In a study two types of stimuli were used: Blue circles on the walls in hazardous areas (elevators, exits, etc) and orange bars in areas where ambulation was encouraged (lounges, hallways, etc). Prior to posting of the stimuli, each patient received a training session of 20 presentations of each stimulus, during which time the orange bar was paired with a favored food and the blue circle was paired with aloud hand clap. After this intervention wandering decreased from 8.7 entries per 30 min to <1.

2. Aggression and agitation: There are some recommended strategies which are widely accepted: (1). Intervene early, before it becomes a crisis. (2). Keep the patient away from provoking situations. (3). Use a reassuring and gentle voice, (4). Approach with a low and calm way from the front, (5). Use touch judiciously, (6). Use nonthreatening postures, (7). Turn their attention to something pleasant, (8). Avoid arguing and trying to reason, physical restraint. For example: If a person with dementia does not recognize his own mirror image and reacts aggressively, many cares turn mirrors to face the wall.

3. Verbal aggression: An example: Spayd and Smyer, 1988 reported a case of a confused elderly man whose verbally aggressive behavior and vulgarities resulted in laughter from his fellow-resident audience. The behavior treatment ion this case included having all staff and patients ignore the inappropriate verbalization, while at the same time increasing attention to the patient whenever he made pleasant statements. Another example reported by Smith, 1988 was about a male AD patient and his wife who would frequently end up in an argument when she attempted to answer his repetitive questions with accurate but complex, explanations. In treatment , the wife was instructed to keep the answers simple and concrete, by using "cue cards" containing one sentence answers to the most frequently asked questions. Each time the question was asked by the patient, his wife made no response except to hand him the appropriate "cue card".

4. Depression: Depression and apathy can be helped by the provision of constructive activity. Everybody can increase and encourage activities that the patient can enjoy. Those that the person enjoyed in the past. These interventions might be appropriate as an adjunct to pharmacotherapy, or when drugs are contraindicated. Ter and Galagher, in 1992 reported that treatment of those patients with mild impairment using a cognitive therapy approach. Based on the idea that depression results from persistent negative distortions about one's self and one's future. The goal of cognitive therapy is to teach the patient to recognize distortions and generate more appropriate and adaptive views of one's life.

Evaluation of a community-based psychoeducational -nursing intervention designed to teach home caregivers to manage behavioral problems of persons with AD using the Progressively Lowered Stress Threshold model and comparison with routine information and referrals for case management , community based services and support groups. The result was a decrese of depression among caregivers who received the training (Buckwalter KC et al, 1999).

5. Social isolation: Social isolation contributes to depression and lack of motivatiuon to maintain personal hygiene, Castensen and Erikson in 1986 reported that refreshments in the social area of home increased the interactions. In developing interventions that teach AD patients how to communicate, therapists should look at the correlates of social isolation, such as depression, social anxiety and mental status.

6. Inappropriate sexual behavior: There are some recommended strategies: If a patient is addressed then you can calmly bring hi or her a robe and matter-of-factly, help him or her to put it on. If the patient is found to masturbating, try not to be act upset or distressed and avoid confrontations, as this may precipitate a catastrophic reaction. Gently lead the person to a private place. Distract the patient by providing something else to do. Prevent future episodes by increasing involvement in ongoing activities.

7. Psychological interventions: These interventions including psychotherapy (individual, group, and family)may be useful, particularly in the early stages of dementia.

8. Other interventions: Recreational, music, pet, art, dancing and bright-light therapies are interventions that have been shown to reduce anxiety and agitation in other populations and which are sometimes used with dementia patients.

9. Combination of interventions: Physical activity and antidepressant treatment have each separately been of significant interest for the management of AD. Particularly the behavioral problems associated with this dementing disorder. This combination for 20-day period led to a signioficant potentiation of full length BDNF mRNA levels within the dentate gyrus abd CA1, CA3, CA4 cellular fields of rats, above the levels obtained with each intervention alone (Russo-Neustadt, A. et al, 1999).

D. Environmental interventions

People with dementia commonly develop symptoms such as agitation, aggressive behavior, delusions and sleep disturbances. Major tranquillisers are commonly prescribed to relieve behavioral difficulties. The side-effects of major tranquillisers

include over-sedation, stiffness, shaking, constipation and falls. Even newer treatments, such as risperidone, olanzapine or quetiapine – usually referred to as atypical antipsychotics – may cause side-effects, though fewer. It is well known that there is a clearly place for drugs in treating behavioral problems but the decision has to be taken carefully and the following points considered: (1). Every caregiver has to find enough space for the person with dementia to walk around. (2). Soft background music and an appropriate level of lighting can be helpful. (3). Signs to help a person to orientate in an environment can reduce restlessness and distress. It is obvious that there can be a need for drugs at certain stages of dementia but it is important to remember that the disease process is following a path and any treatments should be monitored and adjusted accordingly.

Patients with dementia may become fearful, anxious or restless because they cannot properly see or hear their environment. Thus patients need to be examined routinely to evaluate vision and hearing. Although definitive data are lacking, the following environments may be considered for patients with dementia: (1). Special care units within long -term care facilities. (2). Homelike physical setting with small groups of patients as opposed to traditional nursing homes. (3). Short term, planned hospitalization of 1-3 weeks with or without blended inpatient and outpatient care. (4). Provision of exterior space, remodeling corridors to simulate natural or home settings, and changes in the environment. The ideal environment for a patient with dementia is one that is non-stressful, constan and familiar.

E. Interventions for caregivers

All the above mentioned programs can be organized for both caregivers and patients. There are also programs only for caregivers. Since 1987, the New York University Alzheimer's Disease Center Caregiver Research Program has evaluated the benefits of a multifaceted, structures treatment program for spouse-caregivers of patients with Alzheimer's Disease. This intervention strategy for caregivers was based on the clinical experience of counselors who worked with families of AD patients both individually and in support groups for many years. The unifying theme of the intervention is that improving social support and mastery improves the ability of the caregiver to withstand the difficulties of caregiving. The intervention takes into account the diversity and variability in caregiver problems, and is not time limited.

Mittelman MS reported that their treatment program for caregivers consisted of three components: (a) individual and family counseling sessions tailored to each caregiver's specific situation; (b) weekly support group participation; and (c) continuous availability of counselors to caregivers and families to help them deal with crises and with the changing nature of the patient's symptoms over the course of the disease. An important component of the intervention is the involvement of other family members in addition to the spouse[37].

The following interventions may benefit caregivers of persons with dementia and may delay long-term placement: (1). Comprehensive psychoeducational caregiver training, (2). Support groups.

There are many studies about psychological approaches of caregivers to coping with stress Some tips for managing stress are: (1). Look after yourself. Your well-being is extremely important. Do not neglect your health or your feelings. (2). Try not to be too critical or angry. Being critical or angry nearly always results in feelings of discontent, increases levels of stress and achieves nothing constructive. Try to focus on positive things. Give the person you are caring for praise for success rather than criticism for failure. (3). Keep your expectations realistic. Try to adopt a reasonable approach about how much the persons you are caring for can do for themselves. Don't take over things that they could manage as it will cause resentment on their part and over-burden you. The person may be able to complete a task when it is broken down into sections. (4). Make space for yourself. Do not try to be with the person you are caring for all the time, watching them. Give yourself sometime away, even if you are just in another room. Allow yourself time to do nothing, sit and relax or read a book rather than trying to rush about and do chores. (5). Get help with caring. You must take a break from caring time to time, even if it is just for a couple of hours. You can ask for help from family, friends and neighbours or from social services or voluntary organizations. If people offer help, take it. (6). Talk to other people. Caring for someone with dementia can be a lonely experience. It is important to set time aside to talk to others. Some people benefit from talking to other carers at a support group. (7). Get down to resident's level, Stay to the side, offer your hand-palm up and open, Call the person by name, make eye contact, use few words, offer concrete choices, say it once and count to 10, use praise – "yes", “good”, smile with your mouth and eyes, stay relaxed, support and stabilize movement.

Each caregiver in the treatment group receives all the interventions, and each is provided with support for an unlimited time. A recently completed counseling manual describes the intervention in detail[38]. As we all know also there is also our European Care Manual that is translated in many languages of different countries and is a very great help for European caregivers.

A recent study which compared three different ways to reduce stress showed that the carers receiving the sessions from the clinical psychologist experienced a significant reduc-

tion in the level of stress compared with what they felt before starting the study. There was also an effect on the person with dementia-it was found that there was a decrease in behaviours such as aggression and walking about or wandering.

There was little change in levels of stress for those carers who received a session of discussion while the caregiver had the chance to express their views at a 90 minute session. The third group showed no benefit from the provision of information alone.

Assessment of difficulties in caregiving

Both men and women caregivers have been found to be distressed. In cross-sectional comparisons, female caregivers are more likely than male to report depressive symptoms or affect and suffer more distress as the result of life events[39]. Findings from recent research indicated that caregivers' experiences in multiple roles could both detract from and enhance their mental and physical health[40].

The relations between primary and secondary caregivers can be also examined and be the target of education[41]. Interventions for caregivers have to be continued even after the death of the demented patient. Some studies support that caregiver depression exists until two years after bereavement[42].

Service providers should also be aware that memory and behavior problems appear to be the most troubling aspect of the illness for the majority of caregivers. Again education could help caregivers develop strategies to understand and cope with memory and behavioral problems . Time away from the patient through adult day-care programs, periodic respite care, or home attendant services, may also help alleviate the burden of patient's behavioral problems[43].

Complementary therapies and dementia

There has been a growing interest in complementary medicine in recent years. The term complementary medicine describes a diverse range of therapies. What they have in common is an attempt to treat the whole person, including their psychological the emotional aspects, not the physical symptoms or diagnosis. Complementary therapies do not tackle the cause of the disease or delay its progress, but there are potential benefits for people with dementia and cares. Aromatherapy and massage, for example, have many supporters in dementia care. For people with dementia they may offer reassurance through touch and personalized care and may help alleviate agitation, anxiety and sleep disturbance. For carers such treatments offer ways to de-stress and relax. Reflexology, Nutritional Therapy, Herbalism, Massage, Homeopathy, Acupuncture, Aromatherapy were used to help people with dementia and their caregivers.

Finally, professionals and family caregivers of demented individuals, especially if they have experience in terminal care decisions, strongly favor palliative care for end stage dementia. 819 physician members, 1000 non-physician members of Gerontological Society of America and 500 families of demented relatives chose palliative care only (Lunchins DJ and Hanrahan, 1993).

Governments, local communities, people with dementia and their cares all have a part to play in enhancing quality of life in dementia.

For example, governments can be lobbied to provide a fair level of financial support for people with dementia and their carers. Community health workers can learn new skills in dementia training programs. A person newly diagnosed with dementia and his/her family might benefit from an information booklet. Alzheimer associations work at each of these levels, raising awareness about dementia and what support there is available. A person with dementia continues to be a person of worth and dignity and it is always important to remember this.

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Inflammation in Alzheimers's Disease: its Role and an Opportunity for Therapy

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Abstract

Alzheimer's disease (AD) is associated with a dense distribution of activated glia and elevated levels of inflammatory proteins that may underlie the neurodegeneration. Chronic neuroinflammation in rats, produced by infusion of LPS into the 4th ventricle, induced the following changes: increased numbers of activated microglia in the temporal lobe, degeneration of neurons in the entorhinal cortex; shrinkage of temporal lobe regions that can be observed using MRI; elevated levels of cytokines in vulnerable regions; a spatial memory impairment and impaired LTP in the hippocampus; and the selective destruction of cholinergic neurons in the basal forebrain. No other model of AD has reproduced so many different aspects of AD. Therapy with nitro-NSAIDs reduced brain inflammation, rescued cholinergic neurons and improved memory. Our results further indicate that therapies that are designed to slow the onset of AD should be initiated in genetically predisposed adults prior to the appearance of brain inflammation.

Keywords: *Alzheimer's disease, neuroinflammation, animal models, NSAID, memantine*

Introduction

Alois Alzheimer was the first to notice an inflammatory reaction within the brains of his patients. Recent studies indicate that inflammatory processes play a critical role in the pathogenesis of Alzheimer's disease (AD)[1]. AD is associated with an increase in the level of interleukin-1β (IL-1β) in the brain and cerebrospinal fluid[2]. Neuritic plaques in the brains of AD patients contain both beta-amyloid and activated glial cells that over express neurotrophic cytokines, such as IL-1β and tumor necrosis factor-alpha (TNFα). TNFα is able to trigger the production of beta-amyloid peptides and inhibit the secretion of soluble APPs by human neuronal cells[3]. Beta-amyloid is also able to stimulate microglia to secrete additional cytokines and reactive oxygen species[4]. Overall, brain regions that demonstrate the greatest degree of microglial activation early in the disease process ultimately show the highest rate of atrophy and pathology[5]. The important role of inflammation in the pathophysiology of AD is also supported by reports that patients who chronically take anti-inflammatory drugs have a reduced risk of developing AD[6].

A major hurdle in AD research today is that none of the animal models or hypothesized biological mechanisms either previously or currently in fashion has been able to explain the cellular and regional distribution pattern that characterizes the neuropathology of AD. Aging rats do not develop the typical pathological changes that characterize AD, such as senile plaques and neurofibrillary tangles, and transgenic mice show pathological changes throughout their brains that often do not correlate with either aging or a significant loss of cognitive function[7,8]. In the course of studying the effects of chronic neuroinflammation, it has become increasingly obvious that this process qualifies as a promising candidate mechanism to help explain the patterns of the pathophysiological changes that characterize AD. We have developed an animal model of chronic neuroinflammation, using a chronic infusion of lipopolysaccharide (LPS) into the 4th ventricle of young rats, which reproduces many of the behavioral, neurochemical and neuropathological changes associated with AD[9,10,11]. This chapter will describe recent attempts to study the contribution of chronic neuroinflammation to the selective regional neurodegeneration associated with AD and how this informa-

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tion has been used to design more effective therapies for AD.

The most important point to make at the onset of this review is that neuroinflammation does not cause AD. Rather, the consequences of the neuroinflammation sculpt the pathology. Brain inflammation develops in response to other environmental risk factors or existing genetically determined conditions within brains of AD patients. The extent and degree of the brain's inflammatory response to these conditions is influenced by normal aging and varies in different regions. In AD patients, brain regions that develop the greatest and most prolonged inflammatory response are also those that show the greatest degree of neuropathology[5]. The consequences of neuroinflammation in the AD brain may also underlie the loss of specific neural systems. For example, chronic exposure to inflammatory proteins leads to the selective degeneration of basal forebrain cholinergic neurons[12,13].

Two different animal models of brain inflammation will be discussed. The first involves the chronic infusion of LPS directly into the 4th ventricle in order to produce widespread inflammation throughout the brain. The 4th ventricle was chosen in order to avoid mechanical injury to forebrain structures that might impair the cognitive abilities of the animals. The second approach involves the infusion of LPS, or TNFα, directly into the basal forebrain region. This region was chosen for study because the cholinergic neurons within this structure degenerat in the brains of patients with AD.

Studies of chronic neuroinflammation

We initially investigated the time-dependent evolution of the distribution and density of activated microglia that appear in response to the LPS infusion into the 4th ventricle of young rats. We found that after 21 days of continuous infusion the greatest inflammatory response was concentrated within three brain regions: the hippocampus, particularly the dentate gyrus; the entorhinal and piriform cortex within the temporal lobe; and throughout the cingulate gyrus[12]. These results demonstrated that certain brain regions respond differently to the continued presence of an inflammogen such as LPS. The continued activation of microglia by an inflammogenic molecule could lead to the long-term exposure of temporal lobe neurons to potentially toxic levels of cytokines and complement proteins. These data might explain the apparent concentration of AD-related pathology, i.e. senile plaques and tangles, within the temporal regions and the hippocampus. Recent studies using PET have confirmed that brain regions that demonstrate the greatest inflammatory response also show the earliest detectable declines in glucose utilization[14], particularly in those patients who are vulnerable to AD due to their APOE status[15].

It is important to acknowledge that chronic LPS infusion into the 4th ventricle does not reproduce all of the pathological components of AD. However, the following features that are associated with AD were observed. The brains of these young rats had increased activation of microglia and astrogliosis within temporal lobe structures. The hippocampus and basal forebrain regions had elevated levels of various inflammatory proteins, such as IL-1 (α and β) and TNFα, as well as elevated levels of amyloid precursor protein (APP) production. The glial cell activation and presence of elevated inflammatory proteins was associated with significant temporal lobe pathology, i.e. pyramidal with cell loss[9,12]. Furthermore, and most importantly, these rats also demonstrated impairments in tasks that depend upon an intact hippocampus, e.g. performance in the Morris water maze and a spatial memory task on a T-maze. In contrast, these chronically inflamed rats were not impaired in the performance of tasks that do not require an intact hippocampus, e.g. object recognition[9,16]. The working memory impairment demonstrated in the Morris water maze task correlated with the degree of inflammation within the hippocampal region and could be made worse if the LPS infusion was increased to seventy-four days[17].

The inflammation-induced loss of hippocampal pyramidal cells, as well as other types of neurons[18], may also underlie the impaired maze performance that was observed in rats chronically infused with LPS into the 4th ventricle[9]. MRI studies have identified enlarged lateral ventricles with shrinkage of the temporal lobe regions in rats chronically exposed to the effects of low-level neuroinflammation[10]. The ventricular enlargement and atrophy of temporal lobe regions, which is very similar to that seen in patients with AD in the early phases of the disease[19,20], is likely related to cell loss within temporal lobe structures[9,10].

These findings predict that the effects of inflammation upon working memory could be reversed by treatment with an anti-inflammatory drug. We tested this prediction using a novel nitric oxide-releasing cyclooxygenase (COX) inhibitor, nitroflurbiprofen (NFP, NicOx, France). The anti-inflammatory properties of NFP are due to its ability cross the blood-brain barrier and inhibit both isoforms of the COX (types 1 & 2) enzyme leading to the suppression of prostaglandin synthesis[21]. Addition of a nitroxybutyl moiety to flurbiprofen reduces its ulcerogenic properties without interfering with its ability to suppress these inflammatory processes[22]. The degree of inflammation and the level of inflammatory proteins as well as the impairment in performance in the Morris water maze task were all significantly attenuated in young rats given chronic NFP therapy[16,23]. The positive behavioral effects of chronic NFP treatment were probably due to the inhibition of prostanoid synthesis within activated glia and the subse-

quent decreased production and release of cytokines and complement proteins.

Because AD is a disease that is closely associated with aging, we also investigated whether aged rats could benefit from the chronic anti-inflammatory therapy. Young (3 month), adult (10 month) and aged (24 month) rats were infused with LPS for forty-two days. The aged control (those infused with artificial CSF) rats demonstrated poor performance on the Morris water maze, as compared to young or adult control rats. The performance of adult rats was significantly worse than young rats, but significantly better than old rats. When the brains of young rats were chronically infused with LPS their performance did not differ from that of adult and old rats infused with CSF. Also, in contrast to young rats, the brains of control adult and aged rats had numerous activated microglia. Aged rats had the greatest number of activated microglia. Furthermore, the young and adult rats had a greater inflammatory response following the LPS infusion, as compared to the aged rats. We speculate that the level of inflammation reaches a maximum level with normal aging that the LPS infusion was not able to increase. Overall, the results of these studies are consistent with the hypothesis that performance in the Morris water maze can be impaired by the presence of a chronic inflammatory condition, particularly within the hippocampus[23]. Furthermore, these data suggest that the increase in inflammation that accompanies normal aging[24] might underlie the age-associated impairment in performance in this and other tasks that depend upon normal temporal lobe function.

In contrast to the benefits of chronic NSAID therapy that was seen in young rats, NSAID therapy did not improve the working memory ability of either adult or old rats infused with LPS[23]. The chronic NSAID therapy was also ineffective against the level of activated microglia in the older rats, i.e. the therapy did not decrease the number of activated microglia within the temporal lobe regions[23].

Chronic neuroinflammation and estrogen therapy

AD occurs with greater incidence in post-menopausal women and the increased incidence may be delayed by estrogen replacement therapy (ERT)[25,26,27]. We have recently investigated the interaction of chronic ERT and LPS-induced neuroinflammation in the female rat and contrasted these findings with those described above for male rats[28]. Ovariectomy did not impair water maze performance. However, addition of chronic ERT or neuroinflammation resulted in an impairment that became exacerbated by the simultaneous occurrence of both conditions. Chronic LPS infusion activated microglia and this activation was not reduced by ERT. Intact females receiving LPS infusion were not impaired in the water maze and had significantly fewer activated microglia. Our results are similar to those of recent clinical studies[29] and suggest that chronic ERT in post-menopausal women may exacerbate the memory impairment induced by the chronic neuroinflammation associated with AD. We speculate that the increased vulnerability of ovariectomized female rats to the effects of chronic estrogen and chronic neuroinflammation may be due to the presence of chronic, rather than fluctuating, estrogen.

Surprisingly, intact females given chronic infusion of LPS into the 4th ventricle were not impaired in the water maze task. This finding is in marked contrast to male rats that received the same treatment of LPS into the 4th ventricle, as described above. The decreased vulnerability of intact females to the effects of LPS infusion may be due to the differential degree of the inflammatory response by brain microglia as well as a differential pattern of the activated microglia. It is interesting that the reduced number of activated microglia in intact animals appears to be localized to extra-hippocampal regions, as there is no difference in the number of activated microglia seen in the dentate gyrus of the hippocampus between ovariectomized and intact animals receiving LPS. Clearly, the influence of gender upon the impact of chronic neuroinflammation remains to be determined.

Studies of inflammation within the basal forebrain

Consistent with the presumed role of inflammation in the pattern of neurodegeneration observed in AD brains is the recent finding that infusion of LPS into the nucleus basalis magnocellularis within the basal forebrain selectively destroyed cholinergic cells in a time-, but not dose-, dependent manner[30,31]. Similarly, the chronic infusion of IL-1β or TNFα also destroyed cholinergic neurons within the nucleus basalis region[13]. The forebrain cholinergic system may be vulnerable to elevated levels of inflammatory proteins, particularly to TNFα. Stimulation of TNFα receptors may induce cell death by "silencing of survival signals" via the inhibition of insulin-like growth factor-1-mediated signaling within neurons[32]. TNFα can also inhibit glutamate re-uptake into astrocytes and may potentiate glutamate receptor mediated toxicity[33-35] within the basal forebrain, a region that is vulnerable to excess glutamatergic function[13].

AD is characterized by a forebrain deficiency of acetylcholine[36] that may underlie aspects of the cognitive impairments associated with AD[37]. The mechanism underlying the degeneration of basal forebrain cholinergic cells is unknown. The results of studies using LPS infusions have led us to speculate that the long-term exposure to elevated inflammatory proteins may lead to the selective degeneration of basal forebrain cholinergic neurons in AD[13,30,31]. A potential role for neu-

roinflammation, and the specificity of its effects upon cholinergic neurons, was initially suggested by a study that isolated antibodies from the sera of AD patients that selectively recognized and destroyed basal forebrain cholinergic cells when injected into a rat brain[38]. In addition, head trauma in humans is a significant risk factor for AD[39] and is associated with increased levels of inflammatory proteins[40] and a decline in the number of basal forebrain cholinergic neurons[41]. *In vitro* studies also indicate that brain inflammation may selectively destroy basal forebrain cholinergic neurons[42].

We have recently demonstrated that basal forebrain cholinergic neurons could be rescued from the cytotoxic effects of chronic LPS infusion by treatment with anti-inflammatory drugs[13,31]. We have also extended our understanding of the mechanisms by which chronic neuroinflammation destroys basal forebrain cholinergic cells. We have shown that glutamate receptor antagonists can provide neuroprotection from the effects of chronic neuroinflammation[13,31].

Taken together, the results of these studies are consistent with a role for both prostaglandins and NMDA receptors in the cascade of biochemical processes that lead to the degeneration of cholinergic cells within the basal forebrain region of AD patients.

In a recent study, TNFα was infused into the basal forebrain region of APPswe and nontransgenic control mice for twenty days with the expectation that the presence of the human AD transgene would enhance the loss of cholinergic neurons[43]. Chronic infusion of TNFα significantly decreased cortical choline acetyltransferase activity, reduced the number of choline acetyltransferase-immunoreactive cells and increased the number of activated astrocytes within the basal forebrain. Surprisingly, the presence of the APPswe gene did not enhance the vulnerability of forebrain cholinergic neurons to the effects of chronic neuroinflammation. Furthermore, treatment of these mice with memantine demonstrated that the neurotoxic effects of TNFα upon cholinergic cells do not require the activation of the N-methyl-D-aspartate receptors. In contrast, as described above, we have previously shown that memantine was able to provide neuroprotection to cholinergic forebrain neurons from the consequences of exposure to LPS.

The results of these studies ovide insight into the mechanisms by which neuroinflammation may selectively target specific neural systems during the progression of Alzheimer's disease.

Conclusions

The results of these recent studies draw important parallels between consequences of the neuroinflammation-induced neurodegeneration in young and old, male and female, rats and the neurodegeneration seen in AD patients. These similarities include the pattern of neurodegeneration within the brain, the types of neurons effected, the abnormal expression of proteins, the nature of the cognitive impairments, and the chronic nature of the neurodegenerative process12. The results of the present study suggest that combined chronic administration of anti-inflammatory drugs and an NMDA receptor channel antagonist, such as memantine, might provide significant neuroprotection in the brains of AD patients. These results also suggest that the long-term, low-dose administration of such a drug regimen might significantly attenuate the processes that drive the pathology associated with AD if the therapy is initiated in genetically predisposed adults before these age-associated processes within the brain have a chance to develop.

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Hippocampal Gene Expression Profiles in Memory-Impaired and Memory-Unimpaired Aged Rats

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Abstract

Individual difference is a well known feature of cognitive deficits that occur during brain aging, both in animal and man. However, the molecular and cellular mechanisms involved remain to be fully identified. Aged Long-Evans rats demonstrate marked individual differences in learning abilities and thus provide a unique model to investigate global alterations in gene expression using a cDNA microarray approach. In the present study, 24-25 months old rats were classified as aged memory-impaired (AI), aged moderately-impaired (AM) and aged memory-unimpaired (AU) animals relative to the performance of young rats in the Morris water maze test of spatial memory, a hippocampus-dependent learning task. The level of expression of genes in the hippocampus of the three groups of aged rats was assessed using cDNA microarray. Major differences were observed when comparing hippocampal gene expression profiles in the AI, AM and AU sub-groups. For example, the levels of expression of acetylcholinesterase, syntaxin A and carboxypeptidase E were markedly increased in the AI versus AU groups while the opposite was seen for NMDA receptor 2B, neurexin II beta-A precursor and tyrosine phosphatase-like protein. Differences between the AI and AM sub-groups were particularly evident for apolipoprotein D, gastric inhibitory polypeptide precursor and acetylcholine receptor protein beta 2 subunit (all three increased) while decreases in gene expression levels between AI versus AM rats were noted for brain hexokinase and neurexin II beta-A precursor. While it is still too early to laim that the observed differences in gene expression levels are directly related to altered learning abilities, our results may suggest new research avenues for a better understanding of features associated with cognitive disabilities in aging.

Keywords*: Aging, Gene expression, cDNA microarray, Morris water maze, Hippocampus, Memory*

Introduction

Learning and memory impairments are commonly observed in the elderly. Although cognitive decline does not invariably accompany aging, older people are atgreater risk. Similarly, aged rodents exhibit marked heterogeneity in their learning and mnemonic capacities. Using the Morris water maze (MWM) to evaluate spatial learning, 24-25 months old Long-Evans rats can be categorized as aged memory-impaired (AI), aged moderately-impaired (AM) and aged memory–unimpaired (AU) rats when compared to young (4-6 months) animals[1-4]. The basis for such variability is unknown, but it is well documented that spatial memory abilities involved in hidden-platform MWM learning is hippocampusdependent[4,5] and can be impaired in aging. While several neurotransmitter systems are implicated in spatial learning[7-10], much remains to be established in regard to changes in intracellular mechanisms and gene transcription occurring with age, especially in relationship with cognitive abilities. In the present study, gene expresions in a rather unique cohort of aged rats with (AI, AM) or without (AU) learning deficits was characterized using the MWM were investigated using a cDNA microarray approach[11,12]. Clusters of gene families were found to be differentially

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affected in the 3 sub-groups of aged rats used here suggesting their possible relevance to the maintenance (AU) or not (AI, AM) of adequate learning abilities with age.

Methods

Animals

Male Long-Evans rats (250 retired breeders) were purchased from Charles River (St. Constant, Québec) at 12 months of age and were housed in our vivarium until they were 24-25 months old. Thirty young rats (6 months old) were obtained at 3-4 months of age and were housed for 2-3 months prior to behavioral testing. Animals were housed in groups of two in cages (43x20x20cm) and maintained on a 12:12 light-dark schedule with ad libitum access to food (Purina Lab Chow) and water. Animal care and handling procedures were approved by the McGill University Animal Care Committee and the Canadian Council for Animal Care.

Morris water maze

Spatial memory was assessed using the MWM task[3,4,13-15]. Briefly, the rats were required to find a submerged platform located 2.5 cm below the surface of water (rendered opaque by the addition of skim milk powder) in a 1.4 m diameter pool. Animals use only distal visuo-spatial cues available within the testing room to locate the submerged platform[16]. The rats were given 3 trials of 90 sec per day over 5 consecutive days. Animals were also assessed for motor, visual and motivational deficits on day 6 by raising the platform 2 cm above the surface of the water (visually cued condition). Four aged rats were unable to find the visible platform within 60 sec and were excluded from the study whereas twenty three aged rats died before the MWM trials. Average escape latency and distance were recorded along with swim paths using a video tracking system (HVS, Buckingham, UK). Over training days 2-5, aged rats with average escape latencies that were greater than 2 or less than 0.5 standard deviations from the mean of the young group were considered to be memory-impaired (AI; n=55) or memory-unimpaired (AU; n=59), respectively. Animals whose mean escape latencies felt between these values wer defined as moderately-impaired (AM; n=109) rats. Statistical significance was assessed using ANOVA and independent T-tests with Bonferroni correction.

RNA isolation, hybridization and imaging of atlas arrays

On day 6, rat brains were quickly removed by decapitation and hippocampi dissected, pooled and immediately stored at -80°C until use in RNAlater, an RNA stabilization solution (Ambion, Texas, USA). Total RNA was isolated from the entire samples of each rat group and DNase-I treated according to the Atlas Pure total RNA labeling system user manual from BD Biosciences Clontech (Palo Alto, California). The quality, integrity and quantity of the RNA from hippocampi were measured using UV absorption spectrophotometry and ribosomal RNA bands on electrophoresis.

Probes were generated by reverse transcription using the total RNA and ^{32}Plabeled dATP (Perkin-Elmer Life Sciences, Woodbridge, Ontario).

These probes were hybridized on Clontech Atlas Rat 1.2 arrays according to the Atlas cDNA expression arrays user manual from BD Biosciences Clontech. The array data were generated from three experiments using new membranes each time. After hybridization at 68°C for 24 h, membranes were exposed to Kodak Bio-Max MS film (VWR Canlab, Saint-Laurent-Montréal, Québec) using a Kodak high-energy intensifying screen (VWR Canlab) for at least three exposures per membrane (24 hours to 4 days). Array images were acquired using an MCID System (Imaging Research, Saint-Catharines, Ontario).

Analysis of array data

Images of each array were first imported into AtlasImage software (Ver. 2.01; Clontech) for densitometric measurement of gene- and array-specific parameters taking into account film background. A simple ratio for each gene was then calculated using the normalized intensities and averaged with ratios over replicate array values. Only genes that show important change in hippocampal gene expression profiles were selected. Genes were removed if they show a change of 15% or smaller in expression.

Results

Animals were characterized as aged memory-impaired (AI), aged moderately-impaired (AM) and aged memory-unimpaired (AU) by comparing their performance with that of young (Y) animals during training days 2-5 in the MWM task. The AI group showed longer escape latency when compared to Y, AU and AM animals (fig. 1A). There was no significant change in the mean escape latency of aged and young rats when the platform was visible, suggesting the absence of significant motor, visual or motivational deficits (fig. 1B).

Hippocampal gene expression profile of the three groups of aged rats was then compared using a cDNA microarray approach (Table 1). Several genes are differently expressed between the AI and the two other groups shown here as average simple ratio compared to the AI group. The most upregulated gene is the acetylcholinesterase, which was increased by 36 fold in AI versus AU rats (Table 1). Syntaxin A, SNAP-25, GIP and carboxypeptidase E genes were also among the most over-expressed genes between these two groups of ani-

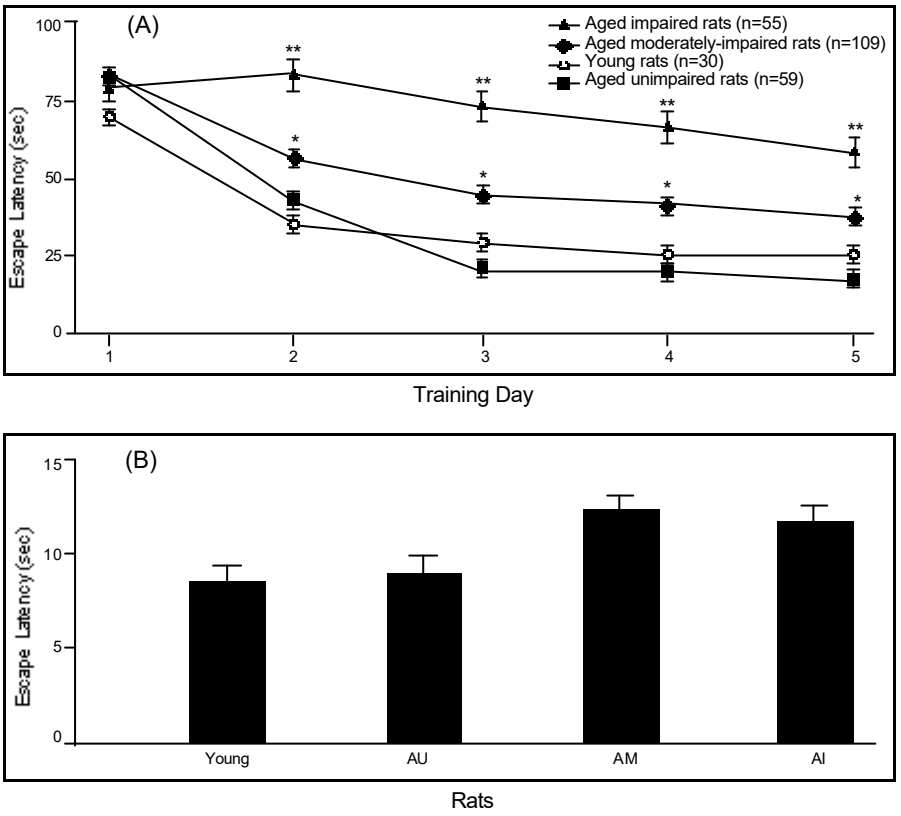

Figure 1. Performances of young (4 mo) and aged (24-25 mo) Long-Evans rats in the Morris water maze behavioral task. (A) Aged-impaired (AI), aged moderately-impaired (AM), and aged-unimpaired (AU) animals were identified as those whose mean escape latencies on training days 2-5 differed by > 2 (AI) or < 0.5 (AU) SD or between (AM) from the mean of young (Y) controls, respectively. * $p<0.05$ and ** $p < 0.01$ compared to young animals, ANOVA and post-hoc t-test. (B) Escape latency when the platform was visible for 60 sec during the cued condition revealed no significant changes among the AI, AM, AU and Y rats, indicating no obvious motivational or visual deficits. All data are presented as mean ± S.E.M of the number of animals indicated in A.

mals. In contrast, gene expressions of NDKA, sodium/potassiumtransporting ATPase beta 1 subunit, brain hexokinase, mitochondrial ATP synthase beta subunit, neuronal acetylcholine nicotinic receptor protein beta 2 subunit, NMDA receptor 2B, neurexin II beta-A precursor, MIF, transducin beta-2 subunit and tyrosine phosphatase-like protein were apparently down-regulated in the AI versus AU animals.

Differences in gene expressions were also noted between the AI and AM subgroups (Table 1).

For example, apolipoprotein D (by 7 fold) and GIP (by 9 fold) expression levels are apparently markedly increased in the AI versus AM animals while the most significant decreases are seen for genes such as brain hexokinase and neurexin II beta-A precursor.

Discussion

The present study confirms that clear individual differences are observed in learning abilities in aged Long-Evans rats with three broad sub-groups being characterized as aged memory impaired (AI), moderately impaired (AM) and unimpaired (AU). Moreover, multiple differences in hippocampal gene expression profiles (as revealed by cDNA microarray) were seen between these three groups of aged animals.

As reported earlier[1-4], marked individual differences were observed in the MWM task in aged Long-Evans rats. Similar differences were noted in other learning tasks including versions of the MWM[17-18] and dry mazes[19-22]. While various neurotransmitters[7-10], neurotrophic factors[13,23-25] and intracellular messengers[26-28] have been studied to possibly explain individual differences in learning abilities in aged rats, much remain to be known as to key cascades of events leading to altered versus maintained cognitive abilities in this model.

A cDNA microarray analysis strategy was employed in the present study to investigate gene expression profile in the hippocampus of AI, AM and AU rats; this region is believed to be critical for acquisition and retrieval of spatial information as well as for memory consolidation and storage[29].

Interestingly, the most upregulated gene in the AI versus AU group was found to be the acetylcholinesterase, the main enzyme that hydrolyzes acetylcholine (ACh). This finding is in accordance with much evidence that have shown the critical role of ACh in the maintenance of normal cognitive functions[10,15]. Morover, acetylcholinesterase inhibitors are still the only drugs approved and widely used for the treatment of Alzheimer's disease.

Another gene that apparently increased substantially was syntaxin A. Its translated protein is known to mediate the docking of synaptic vesicles at the level of nerve terminals leading to neurotransmitter's exocytosis[30]. SNAP-25, another gene implicated in regulated and constitutive exocytosis in neurons[31], was also apparently increased in AI versus AU rats. Changes in gene expression levels of both SNAP-25 and syntaxin A could lead to significantly altered profiles of neurotransmitter releases. Other gene expressions that were apparently increased in the AI sub-group include GIP and carboxypeptidase E. The role of peptide GIP in the brain is mostly unknown while carboxypeptidases are known to be involved in the mat-

Table 1. Gene expression pattern observed in the hippocampus of aged memory-impaired (AI), moderately-impaired (AM) and memory-unimpaired (AU) rats.

Genbank number	Gene	AI / AM	AI / AU
D38492	neural adhesion molecule F3	2.40	0.29
M20035	prothymosin-alpha (PTMA)	3.38	2.54
X55572	apolipoprotein D	7.02	2.09
D13374	nucleoside diphosphate kinase A (NDKA)	0.88	0.14
X06942	A-raf proto-oncogene	1.85	0.46
J02701	sodium/potassium-transporting ATPase beta 1 subunit (ATP1B1)	1.24	0.07
M95734	syntaxin A	2.10	13.57
M95735	syntaxin B	1.48	2.02
AB003991	synaptosomal associated protein 25 (SNAP-25)	0.93	3.87
J04526	type 1 hexokinase (HK1); brain hexokinase	0.16	0.10
M19044	mitochondrial ATP synthase beta subunit (ATP5B)	0.96	0.14
S50879	acetylcholinesterase (ACHE)	0.42	36.46
M27905	60S ribosomal protein L21 (RPL21)	1.19	4.23
J02650	60S ribosomal protein L19 (RPL19)	1.05	4.19
L31622	neuronal acetylcholine receptor protein beta 2 subunit (NACHRB2)	3.87	0.40
M91562	NMDA Receptor 2B	0.96	0.08
AF005099	neuronal pentraxin receptor	2.35	0.42
M96377	neurexin II beta-A precursor	0.43	0.04
U62326	macrophage migration inhibitory factor (MIF)	0.86	0.16
L08831	gastric inhibitory polypeptide precursor (GIP); glucose-dependent insulinotropic polypeptide	9.71	4.40
M64300	extracellular signal-regulated kinase 2 (ERK2); mitogen-activated protein kinase 2 (MAPK2)	3.76	Nd
M16112	calcium/calmodulin-dependent protein kinase type II beta subunit (CAMK2B)	0.96	0.38
U34959	transducin beta-2 subunit; GTP-binding protein G(i)/G(s)/G(t) beta subunit 2 (GNB2)	0.76	0.08
D13125	neural visinin-like protein 2 (VILIP1; VSNL1)	1.98	2.56
M16736	growth-accentuating protein 43 (GAP43); neuromodulin	1.80	2.94
M31602	carboxypeptidase E (CPE)	1.30	5.32
L19181	receptor-linked protein tyrosine phosphatase (PTP-PS)	1.91	2.89
D38222	tyrosine phosphatase-like protein	1.10	0.05

Hippocampal cDNA array gene expression levels reaching criteria for changes sorted by average simple ratio following median normalization. Array hybridization was repeated three times. For gene grid assignment, refer to BD Atlas Rat 1.2 Array Gene List (PT3564-3) web site (http://www.clontech.com/atlas/genelists/7854-1_Ra12.pdf). For cDNA gene database, refer to BD Atlas gene lists version 5.0 (PT3593-CD) web site (http://atlasinfo.clontech.com/search/initialization-action.do). Nd, not determined.

uration of various peptide precursors and other metabolic activities[32,33]. Further studies will be required to establish if they may play a direct role in cognitive functions.

Conversely, the level of the gene coding for neurexin II beta-A precursor decreased most significantly in AI compared to AU rats. Neurexins are neuronal cellsurface receptors that likely perform multiple functions in the brain by participating in intercellular junctions and by acting as signaling receptors[34]. Interestingly, mRNA levels of neurexins II alpha and III alpha were found to be upregulated in CA1 and CA3 hippocampal neurons in response to a 15-min ischemic period[35]. The apparent level of the gene encoding for the tyrosine phosphatase-like protein level also decreased in AI versus AU rats. This membrane protein is expressed only in the brain and may function as a negative regulator of PTPases[36].

Other genes of interest included those coding for the NMDA receptor 2B, the nicotinic receptor protein beta 2 subunit, sodium/potassium-transporting ATPase beta 1 subunit, brain hexokinase and mitochondrial ATP synthase beta subunit. Much evidence has shown the important roles of NMDA[14,37,38] and nicotinic[8,39] receptors in learning abilities. Similarly, altered cellular metabolic functions associated with the other genes mentioned above could

impact on the maintenance of appropriate learning abilities. Further studies are currently underway to verify this hypothesis.

Fewer genes apparently had modified levels of expression between the AI and AM sub-groups. This could be expected as learning abilities in the AM group is significantly but not dramatically distinct from that of the AI rats. Of particular interest is the up-regulation of GIP in the AI versus AM animals. As noted above, a similar increase was also seen between the AI and AU sub-groups suggesting that further investigations of this gene and its translated peptide are certainly warranted. Among the genes that are apparently most significantly down-regulated in the AI versus AM rats, neurexin II beta-A precursor and brain hexokinase are of particular interest as similarly affected in the AI versus AU sub-groups.

In summary, distinct profiles of expression of genes were observed in aged rats with varying degrees of learning abilities as monitored in the MWM task. While it is not possible to demonstrate that these changes at directly related to altered cognitive behaviors at this early stage in our studies, the present results suggest possible novel targets (GIP, hexokinase, neurexin II, etc) that could be considered as an attempt to better understand the molecular origins of differential learning profiles of AI, AM and AU rats.

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Development of mtDNA-Transfused Cell Model of Alzheimer's Disease and Application in Pharmacological Study on Chinese Herb Components

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Abstract

Objective: ***To develop mtDNA-transfused cell model (cybrids) of Alzheimer's disease(AD), observe the pathological characteristics of cybrids, and investigate the pharmacological effects of Chinese herb component SSY-P3 on the cybrid model.***

Methods: ***The platelates were isolated from 6 AD patients,6 normal aged persons, and 6 normal young persons. The ρ_0 cells without mitochondria were fused with the platelets from the donors. Cytochrome oxidase (COX) activity was determined by microplate assay. The specific fragment of mtDNA was detected by PCR and gel electrophoresis. Reactive oxygen species(ROS), mitochondrial membrane potential (MMP) and cytosolic calcium were determined by laser scanning confocal microscopy and flow cytometry.***

Results: ***(1) COX activity in AD cybrids decreased compared with aged and young control; (2) MMP of AD cybrids was lower than that in aged and young control. SSY-P3 Incubation elevated their MMP by 80.4%. (3) ROS in AD cybrids was higher than that in aged and young control. Incubation of Chinese herb component SSY-P3 (100μg/ml) with AD cybrids for 24h declined their ROS production. (4) In comparison of young control, the basal cytosolic calcium of AD cybrid was increased, and the regulatory ability to calcium was decreased. SSY-P3 decreased basal cytosolic calcium and enhanced regulatory ability to calcium in AD cybrids.***

Conclusion: ***mtDNA transfused cells (cybrids) of AD can be used as a good model to investigate the pathogenesis related to mitochondria and to screen the drugs. Chinese herb component SSY-P3 may be beneficial to treatAlzheimer's diseases.***

Keywords: *Alzheimer's disease, mtDNA-transferred cells, cytochrome c oxidase, reactive oxygen species, mitochondrial membrane potential, cytosolic calcium.*

Introduction

Alzheimer's disease (AD) is a commonly occurring and devastating neuro-degenerative disorder whose causes are not known clearly. Mounting evidence suggests that mitochondrial dysfunction is prominent in AD and may underlie the "sporadic" cases of the disease[1]. A mitochondrial defect of complex IV or cytochrome c oxidase (COX) of the electron transport chain (ETC) is found in multiple tissues, including brain, in sporadic AD[2]. Transfer of platelet mitochondrial DNA (mtDNA) from AD patients into clonal host cells (creating cytoplasmic hybrids, or "cybrids") recreates the COX defect in these cells, demonstrating its mtDNA origin[3-4]. As these cells are reproduced by mtDNA mutation of AD patients, cybrids represented early pathological changes of the diseases more exactly than traditional chemical-damaged models. They would be useful in mechanism research and drug screening of AD[5-6].

SSY-P3 is a monomer extracted from Chinese herb SSY (can not make public now because of the patent application for National Patent Bureau) by phytochemistry under pharmacodynamics direction. In our previous study, this compo-

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nent is effective in several neuro-damage models both in vitro and in vivo. The purpose of our present research is to develop AD cybrids as the disease model, observe the pathological characteristics of the cybrids, and investigate the pharmacological effects of P3 on the cell models.

Materials and methods

1. **Case selection:** 6 AD patients diagnosed by the neurologists, without family history of AD, without other diseases of nervous systems; 6 young healthy controls, 6 aged healthy controls.
2. **Cell culture preparation:** mtDNA -deficienct 143B.206 ρ_0 cell line (the derivative of human cell line 143B.TK$^-$ (mtDNA-complete) was donated from NIH[7]). Platelets were isolated from anti-coagulated venous blood and fused with ρ_0 cells under the induction of PEG1500. The unfused ρ_0 cells were removed by replacing the medium with selective (uridine-free) medium. Cybrid clones were visible 10-15 days after fusion[8].
3. **Cytochrome oxidase (COX) activity** was determined by microplate assa[9].
4. **mtDNA detection**: the specific fragment of mtDNA was detected by PCR and gel electrophoresis[10].
5. **Determination of cytosolic calcium**[11-12]: Basal and CCCP (an uncoupler of mitochondrial oxidative phosphorylation which releases sequestered mitochondrial calcium stores)- stimulated cytosolic calcium was determined by laser scanning confocal microscopy (LSCM) and flow cytometry (FCM), using the fluorescent Ca^{2+} sensitive indicator Fluo-3/AM. The increasing rate of calcium after CCCP stimulation were computed from fluorescence intensity ratio using the following equation:

$$[Ca^{2+}]_i(\%)=(F_{max}-F_0) / F_0 \times 100\%$$

where F_0 is the point at which the cell achieved a stable fluorescence baseline, and F_{max} is the top point at which the cell achieved a maximal fluorescence iintensity by CCCP stimulation.

6. **Measurement of reactive oxygen species (ROS):** The levels of 2',7'-dichlorofluorescin fluorecence (DCF-DA, from Sigma), the ROS specific fluorescent indicator, were determined by LSCM and FCM[13-14].
7. **Mitochondrial membrane potential (MMP) assay**: Rhodamine 123 (from Sigma), the specific fluorescent indicator of MMP, were incubated with cybrids for 30 minutes and determined by LSCM and FCM[15].
8. **Extraction and purification of the Chinese herb SSY**: SSY F6 extracted by n-Butanol was separated to 5 fragments (F1-F5) by high-speed countercurrent chromatography (HSCCC). Among them, F3 was confirmed to be effective in 3 neuro-damage cellular models and was further separated to 5 peaks (P1–P5) by HSCCC. P3 is monomer and the effective component among them. When purity determination and structure identification of P3 were completed, we found its purity is above 90% and its molecular weight is about 128 (Figures 1,2).

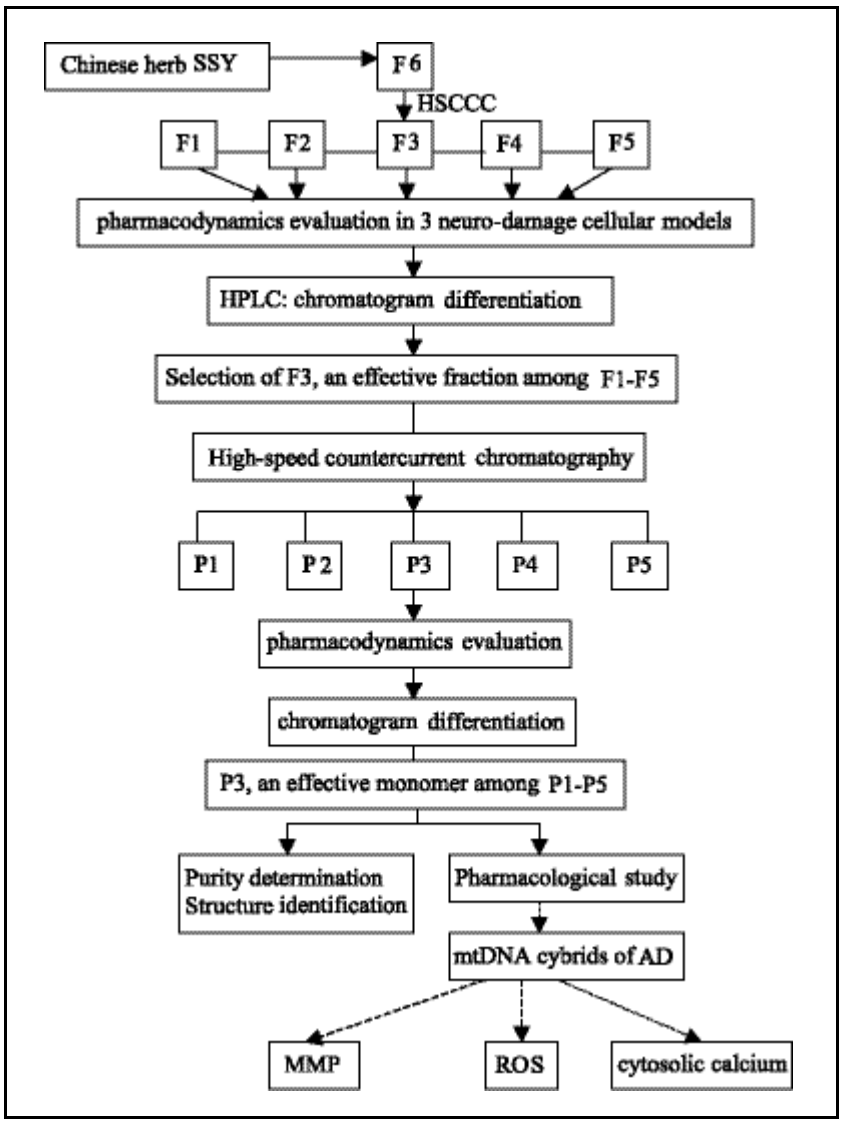
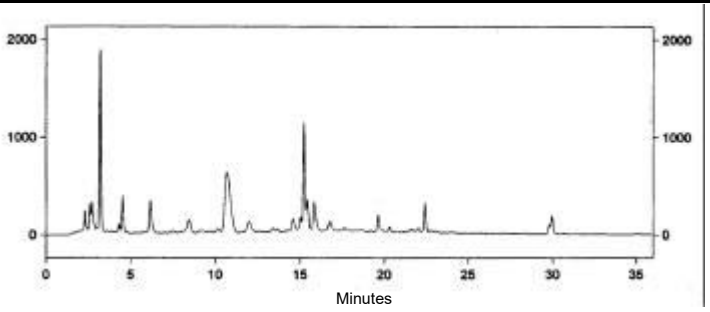
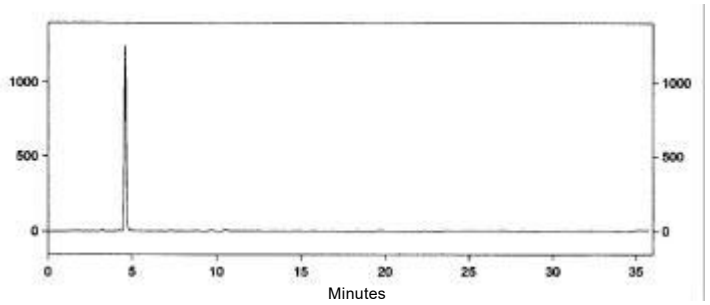


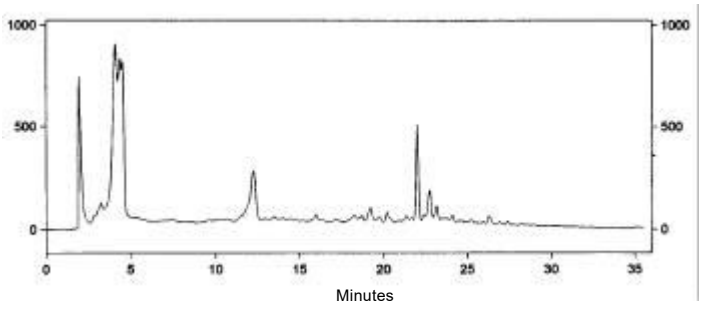
Figure 1. Extraction and purification of SSY under pharmacodynamics direction.



F6 Extraction of SSY in n-butanol.



F3 Isolated from F6, an effective component among F1-F5.



F3 Purified from F3, an effctive monomer among P1-P5.

Figure 2. HPLC analsys of SSY.

9. **Treatment of SSY-P3 in AD cybrids**: While we observed the effect of P3 on some neuro-damage cell models, especially on sodium azide (specific inhibitor of COX, to mimic the mitochondrial deficiency chemically) treated 143B.TK$^-$ cells in the previous work, we found that P3 showed the protection on most of them and the most effective dosage of P3 was about 100 μg/ml P3 for 24 hours. Because the procedures of the experiments were quite complicated, we could only select one dosage to study the pharmacological effects of P3 on AD cybrids.

Results

1. The development and identification of AD mtDNA-transferred cells

The ρ_0 cells without mitochondria were fused with the platelets under the induction of PEG 1500. The unfused ρ_0 cells were removed by replacing the medium with selective (uridine-free) medium. Cybrid clones were visible 10-15 days after fusion (Figure 3).

ρ_0 cells were uridine-dependent and died gradually in selective medium, while cybrids grew and proliferated well in selective medium (Figure 4).

ρ_0 cells had no detectable cytochrome c oxidase (COX) activity, while cybrids recovered COX activity following introduction of exogenous mitochondria. On the other hand, COX activity of AD cybrids decreased 29.1% compared with age-match control and decreased 45.7% compared with young control; aged control was 23.3% lower COX than young control (Table 1).

ρ_0 cells genome contained no objective fragment of mtDNA by PCR and gel electrophoresis; oppositing to that, cybrids had the mtDNA-specific fragment as same as that in 143B. TK$^-$ cells (Figures 5,6). The successful development of cybrids was verified by well growth and proliferation of cybrids in selective medium without uridine, recovered COX activity and the mtDNA–specific fragment amplification.

2. Protective effects of SSY-P3 on AD cybrids

2.1. Effects of P3 on cytosolic calcium

Basal cytosolic calcium of AD cybrids was elevated in comparison of young control. As to CCCP-stimu-

Table 1. Cytochrome c oxidase activity in cybrids.

Group	n	COX (ratio)
143B (normal mtDNA)	6	1.000±0.152*
$\rho_0$206 (no mtDNA)	6	0.021±0.031
$\rho_0$206+Young mtDNA	6	1.055±0.203*
$\rho_0$206+Aged mtDNA	6	0.812±0.215*
$\rho_0$206+AD mtDNA	6	0.573±0.374*#

P<0.05 compared with $\rho_0$206, P<0.05 compared with young cybrids, data are mean ± SD. COX activity is expressed as a ratio of the mean 143B value. Each cybrid line was grown up and assayed independently at least twice.

Table 2. Effects of SSY-P3 on cytosolic calcium of AD cybrids.

Group	n	Basal Ca^{2+}(ratio)	CCCP-stimulated Ca^{2+}(ratio)
Young cybrides	6	1.212±0.348	0.988±0.576*
Aged cybrides	6	1.513±0.802	0.751±0.382*
AD cybrides	6	1.638±0.946	0.353±0.181
AD cybrides +P3	6	1.232±0.593	0.602±0.101*

Calcium was determined as ratio of Fluo-3 fluorescence normalized by the mean 143B. TK^- value. Each cybrid line was grown up and assayed independently at least twice.
* $p<0.05$ compared with AD cybrids; data are mean ± SD.

lated calcium showing calcium storage and modification by mitochondria, AD cybrids were 64.1% lower than young control; aged control cybrids was 23.6% lower than normal young subjects (Figures 7,8). AD cybrids treated by 100 μg/ml P3 for 24 hours showed decreased basal cytosolic calcium of AD cybrids, Meanwhile, P3 increased the peak of cytosolic calcium after CCCP stimulation, suggesting that P3 improved calcium storage of mitochondria (Figure 9,Table 2).

2.2. The effects of P3 on cellular ROS production. It showed that ROS of AD cybrids was 63.5% higher than that of young control, and 44.9% higher than that of aged control; The difference between young and aged subjects was not significant. P3 decreased ROS production of AD cybrids by 24% (Figure 10, Table 3).

2.3. The effects of P3 on MMP. MMP of AD cybrids was 48.7% lower than that of young control and 46.5% lower than that of aged control. There was no significant difference between young and aged control in MMP. AD cybrids incubated with 100μg/ml P3 for 24 hours showed elevated MMP production of AD cybrids by 80.4% (Figure 11, Table 4).

Table 4. Effects of SSY-P3 on MMP of AD cybrids.

Group	n	MMP (ratio)
Young cybrides	6	1.000±0.181*
Aged cybrides	6	1.966±0.285*
AD cybrides	6	1.512±0.205
AD cybrides +P3	6	0.928±0.351*

MMP was determined as the ratio of Rh123 fluorescence normalized by the mean 143B. TK^- value.
* $p<0.05$ compared with AD cybrids; data are mean ± SD.

Discussion

Accumulated evidence suggests that electron transport chain (ETC) defects derived from mitochondrial DNA (mtDNA) play an important role in the pathogenesis of Alzheimer's disease (AD)[16]. Because ETC is double-controlled hereditedly by both nuclear DNA and mtDNA, it is difficult identifying the source of a newly-described ETC defect. A new technique developed recent years may resolved this problem. mtDNA-depleted cells (ρ_0 cells) can be repopulated with exogenous mitochondria derived from patients who may exist mtDNA defects. The only difference between mtDNA-transferred cells (also called cytoplasmic hybrids, in brief cybrids) of patients and those of normal subjects comes from their mtDNA, therefore nuclear DNA and other factors in cytoplasm are excluded, and the role of mtDNA can be investigated individually[17,18].

Table 3. Effects of SSY-P3 on cellular ROS production of AD cybrids.

Group	n	ROS (ratio)
Young cybrides	6	0.922±0.443*
Aged cybrides	6	1.045±0.382*
AD cybrides	6	1.508±0.296
AD cybrides +P3	6	1.144±0.332

ROS was determined as the ratio of DCF-DA fluorescence normalized by the mean 143B. TK^- value.
* $p<0.05$ compared with AD cybrids; data are mean ± SD.

In our study, the successful development of cybrids is verified by the evidence that cybrids grew and proliferated well in selective medium without uridine, recovered COX activity and had the mtDNA – specific fragment; but ρ_0 cells fail. Because the defects of mtDNA derived from AD patient are expressed in the new cell background after fusion, the defective ETC is synthesized in cybrids. A series of pathological events follow including the elevation of reactive oxygen species (ROS) production, the depression of mitochondrial membrane potential (MMP), and the calcium homeostasis imbalance. Our data demonstrate that all of these abnormalities are specific in

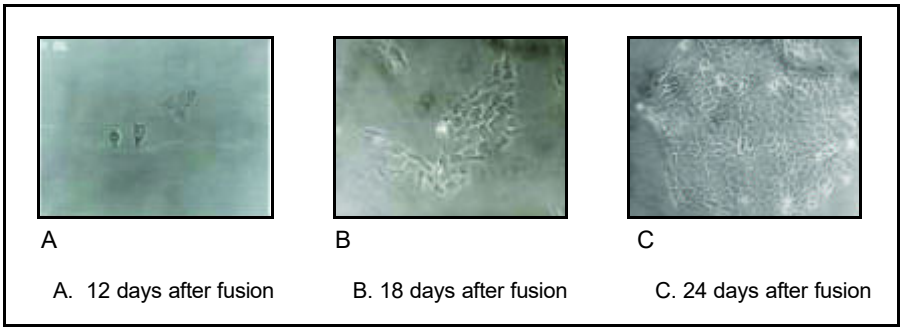


Figure 3. Cybrids clone cultured in selective media

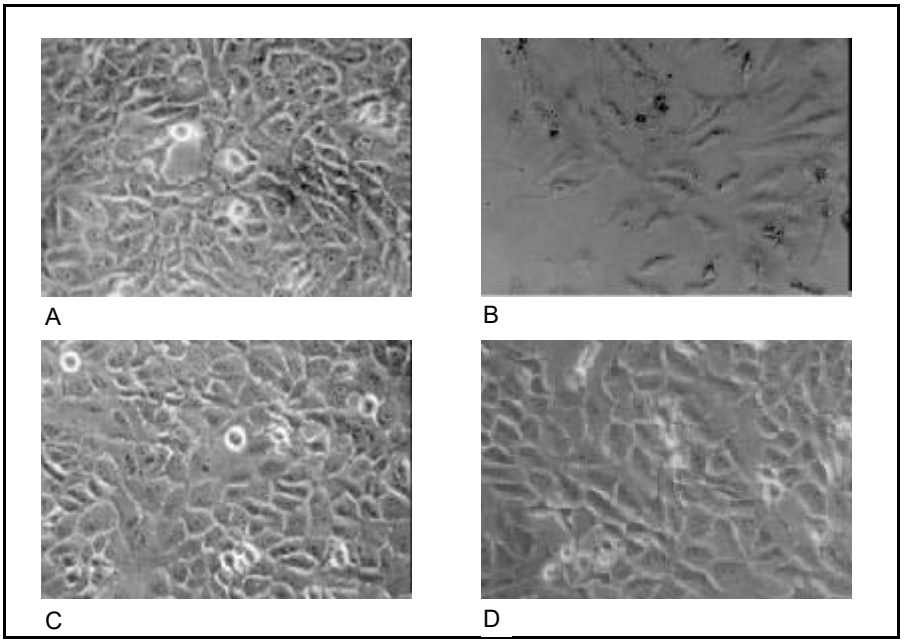


Figure 4. Identification of the successful creation of cybrids by the caracterization of nutrient requirement.

A. ρ_0 cells in normal medium (containg 50 mg/ml uridine)
B. ρ_0 cells in selective medium (without uridine)
C. cybrids in normal medium (containg 50 mg/ml uridine)
D. cybrids in selective medium (without uridine).

AD cybrids compared with aged controls. We believe that decreased mitochondrial COX activity induce the dysfunction of mitochondria (MMP) and has definite functional consequences in terms of calcium handling and ROS production. Because the intromitochondrial calcium concentration is usually much larger than that in the cytosol, CCCP, an uncoupler of mitochondrial oxidative phosphorylation, causes a transient increase in cytosolic calcium and decrease in mitochondrial calcium store[19]. AD cybrids seldom show the significant increase in cytosolic calcium after CCCP-stimulation while they represent higher basal cytosolic calcium compared with aged and young control. These indicate both the intramitochondrial calcium storage and the calcium homeostasis modified by mitochondria are impaired in AD cybrids. For mtDNA defect of cybrids are transferred from AD patients, mitochondrial dysfunction and its functional consequences probably contribute to the pathogenesis of sporadic AD. AD cybrids represent early pathological changes of the disease more exactly than traditional chemical-damaged models, we take the AD cybrids as an AD-like cell model and use it in mechanism research and drug screening of AD.

The monomer SSY-P3 from Chinese herb decrease cellular ROS, elevated MMP, reduced basal cytosolic calcium and increased calcium storage of mitochondria in AD cybrids, therefore improved pathological disturbance in AD cybrids. Neuronal survival may depend on a delicate balance between nuclear and mitochondrial genome stability, mitochondrial function, calcium homeostasis, ROS production and other factors[20]. The actions of P3 may occur initially at the level of the mitochondria and most likely improve ETC and energy metabolism. The ability to protect the mitochondria and prevent the sequent dysfunction of ROS production and calcium homeostasis in AD cybrids by P3 suggests a potential role for this herb extract in AD therapy because these abnormality usually appear in the early phase of AD.

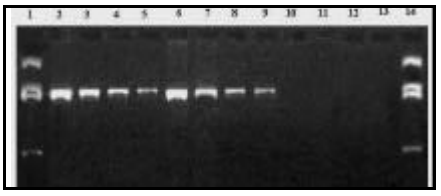


Figure 5. PCR amplification of mtDNA of 143B.TK$^-$ cells line 2-5, ρ_0 cells line 6-9 and cybrids line 10-13.

1,14	DNA markers pBR322 DNA/BstN I Markers
2,6,10	Concentration of template: 200ng/25m
3,7,11	Concentration of template: 40 ng/25m
4,8,12	Concentration of template: 8 ng/25m
5,9,13	Concentration of template: 1.6 ng/25m

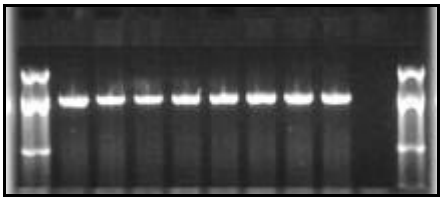


Figure 6. PCR amplification of mtDNA of 143B. TK$^-$ cells, ρ_0 cells and cybrids.

1,11	DNA markers pBR322 DNA/BstN I Markers
2-5	AD cybrids
6,7	cybrids of normal aged control
8	cybrids of normal young control
10	ρ_0 cells

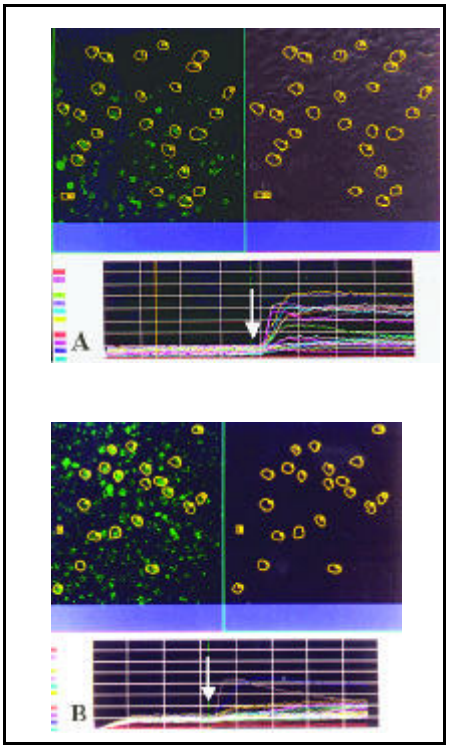


Figure 7. Cytosolic calcium of cybrids determined by LSCM

(top left: Rh123 (green) fluorenscence intensity of basal [Ca^{2+}]i in cybrids;
low curve: Time course of cytosolic calcium in cybrids. Arrow: CCCP stimulation

A. Aged control B. AD cybrids

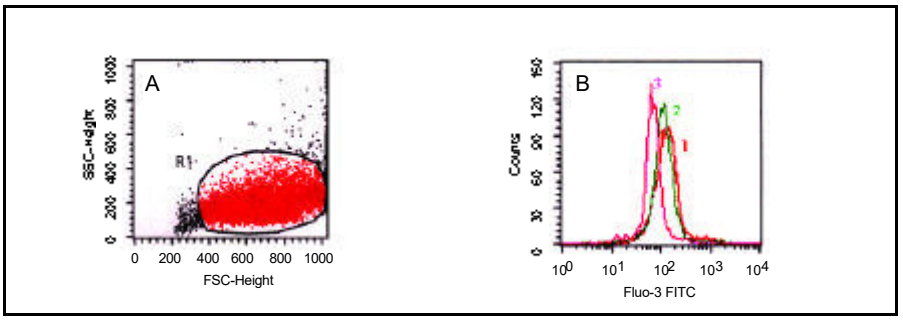


Figure 8. CCCP-stimulated cytosolic calcium of cybrids determined by flow cytometry.

A. Cell mass investigated; B. Fluo-3 fluorenscence intensity of young control, aged control and AD cybrids after CCCP-stimulation the lefter of the curve, the lower of [Ca^{2+}]i. Red(1): young control, green(2): aged control, pink (3): AD cybrids.

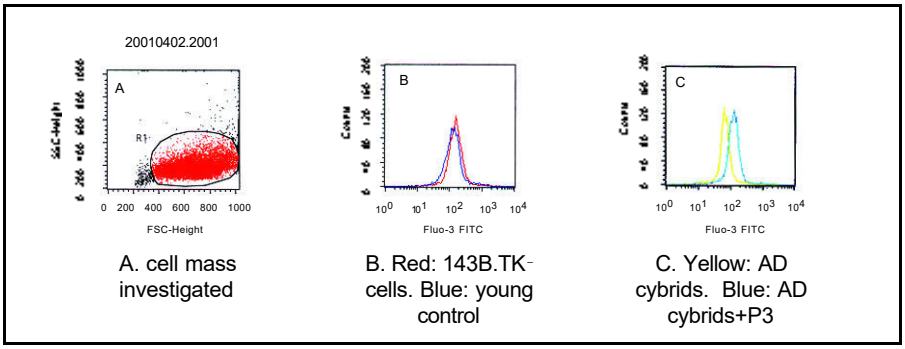


Figure 9. Effect of P3 on CCCP-stimulated cytosolic calcium in AD cybrids determined with FCM.

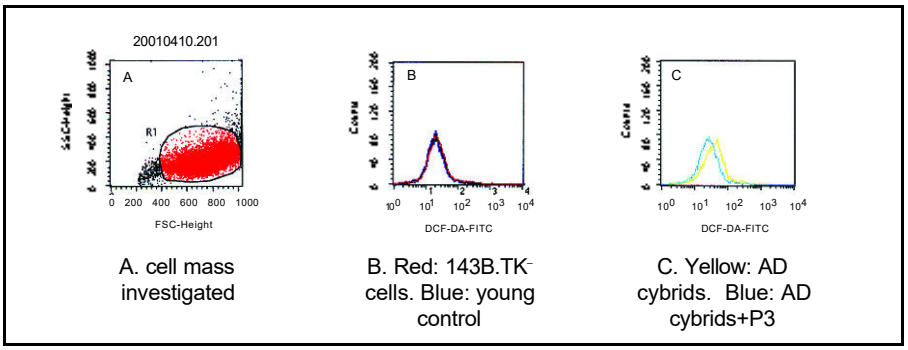


Figure 10. Effect of P3 on Cytosolic ROS production in AD cybrids determined with FCM.

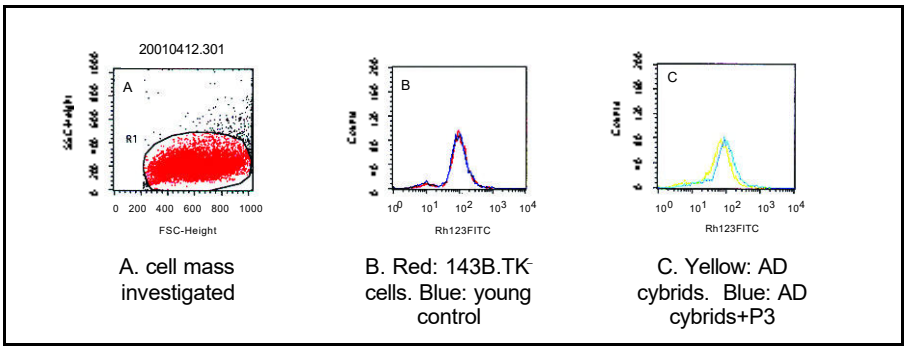


Figure 11. Effect of P3 on MMP in AD cybrids determined with FCM.

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Memory and Language Strategies and their Effects on the Cognitive Abilities in the First Stages of Probable Alzheimer's Disease

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Abstract

Memory and language disorders are of the main deficits in patients with probable Alzheimer's disease (AD) from the first stages of the disease. Memory problems are particularly evident as patients with probable AD cannot retain new information and have naming difficulties. This study represents an intervention program with memory and language exercises in order to train AD patients in the first stages of the disease to use memory strategies for retention of item information as well as of lexical labels. The sample comprised of 10 patients with probable AD as experimental group; they participated in the memory and language training program. There was also a control group of 10 AD patients, matched in terms of age, education, and mental state; they received no memory or language training. The two groups were administered the same neuropsychological tests. The intervention lasted about four months with three hours a week sessions and home exercises for the rest of the weekdays. The results of the intervention showed that AD patients in the first stages of the disease can learn strategies after systematic training and practice and can generalize training to other cognitive abilities, such as attention.

Introduction

Dementia of Alzheimer's type (DAT) has been known to cause word finding deficits since the first stages of the disease. Alzheimer's disease patients express themselves through circumlocutions of the required words, semantic substitutions and frequent pauses in oral speech. Although in the first stages of AD, patients show no phonological paraphasias and preserve automatic speech, in the last stages they are often characterized by the phenomenon of *empty speech*, semantic and/or phonological errors, increased use of pronouns and incomplete sentences[1]. Language deficits in the first stages of AD are very similar to the symptoms of transcortical – sensory aphasia, while in the late stages there are similarities with the symptoms of Wernicke aphasia[2,3,4]. Naming deficits in AD may due to visual – perceptive deficits[5], working memory deficits[6], to semantic memory disorganization[7], or to inability to access the lexical labels of the items[8].

Naming difficulties and the "tip of the tongue" phenomenon are also present in cognitively healthy elderly people; yet picture naming performance can differentiate healthy elderly from AD patients. Studies on picture naming in AD suggest that these patients respond by giving the name of the superordinate semantic category[9].

In so far as the rehabilitation in AD is concerned, most of the early studies that used cognitive support achieved short-term effects after the intervention[10,11,12] and for this reason many researchers talked about the inefficiency of cognitive support[13,14]. However, the first intervention studies did not use patients in the first stages of AD[15]. Also, these studies used various kinds of techniques to enhance memory performance such as organizational instructions, verbal mediators, more time for recall, rich stimuli inputs, or tasks with internal organization and cohesion, or self-generation activity[16]. As the problem in the first stages of DAT did not seem to lie in storage but in the encoding and learning of new information[17], the results were short lived and restricted to the training tasks.

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Furthermore, most studies used cognitive support only for encoding or for recall. However, considering the decline of episodic memory in AD it is necessary to give cognitive support both for encoding and for recall[16].

In recent years there have been many studies that achieved satisfactory results using cognitive support. Thus, there was optimism that cognitive intervention (CI) can reverse the progress of AD[18].
Nevertheless, researchers caution that CI should be designed for each specific type of memory problem, not produce memory load to the patients, and provide the appropriate cues for both encoding and recall processes[19].
Nowadays there are many studies with long term effects and generalization to other psychosocial abilities[20-29]. Also there are studies that used computers in order to provide cognitive support; patients in the studies that used computer games learnt skills that were necessary in everyday life[30,31].
The conclusion from the previous studies is that AD patients in early stages of the disease can learn new information and maintain it for a considerable time. There is still the problem that patients do not transfer strategies and skills they learn to everyday life. This is a criterion for the success of CI.

Our research – The Cognitive Neuropsychological Intervention

The intervention we applied follows the Cognitive Neuropsychoogical Intervention model (CNI). The principles on which CNI is based are given below:

One of the areas of cognitive neuropsychology is language disorders in aphasia and the cognitive processes that are responsible for them (memory, attention, perception, problem solving). Its main aim is to identify the intact and destroyed cognitive processes and representations using lexical tasks. Thus lexical items are used both in the diagnosis and in the rehabilitation of deficient cognitive processes, particularly of problems in verbal comprehension and production of words.

Before the intervention, the examiner selects or designs lexical tasks based on a guiding theoretical model. The lexical tasks are intended to assess the functioning of different language processes. The deficit in language processing can be the result of a problem in one or more stages of the processing. After completing the language tasks, the examiner tries to understand "where" and "how" the normal processing has failed and forms hypotheses about the probable cause of the deficit. In the case of AD the examiner uses semantic tasks such as categorization of items, definition of words, semantic associations and naming, and compares the oral and writing versions of the previous tasks. The type of errors AD patients usually do are visuoperceptual, semantic (use of superordinates, coherence, associational, circumlocution), phonemic, "non-correlated" and "no answers".

The intervention program is considered successful if (a) the posttest scores are significantly better than the pretest scores; (b) if there is generalization to untreated items, and (c) if the model proves useful because of its predictive value as regards language and cognitive processes that are ameliorated through the intervention[32].

The aim of the study

We applied the CNI model to AD patients in order (1) to help patients minimize their word difficulties problems, and (2) to test the generalization of this kind of intervention to other cognitive abilities, such as attention. This is particularly important because cognitive neuropsychological studies are often case studies or aim at representing normal language processing.

Our assumptions

We assumed that the naming deficit in AD is the outcome of a more general deficit that involves not only semantic memory and accessing of the lexical label but also the encoding and retrieval of new information processes. So we assumed that training AD patients with strategies on relearning of faded information or on reorganization of the intact information in semantic memory, will help them retain at least the newly given information regarding the lexical label of the items. Specifically, we assumed that the reorganization memory strategies regarding the function, the characteristics and the name of the objects will make stronger the connections between the semantic and phonological representations of lexical items and will have stronger effects than strategies that focus only on semantic or phonological memory processes.

The hypotheses

1. Teaching of memory strategies on how to keep in memory the lexical labels of the items to be named will support the naming procedure.
2. Memory strategies that facilitate reorganization of existing semantic categories or relearning of the meaning, the function and the lexical label of fading or non intact items will help patients associate the intact information with their lexical labels.

Method

Assessment of DAT

The clinical examination of the participants comprised medical history, biological, physical and neuropsychological tests. Diagnosis of probable AD was made on the basis of NINCDS-ADRDA[34] and DSM IV[35] criteria. We also used the Mini Mental State Examination[36,37] and

the Clinical Dementia Rating[38] scales in order to estimate the general level of cognitive processing and the severity of AD. The Hachinski[39] and Hamilton[40] scales were used for the differential diagnosis of vascular dementia and depression respectively.

The psycholinguistic assessment of language deficits was based on the Boston Naming Test[41], the Pyramids & Palm Trees[42], the Psycholinguistic Assessment of Language Processing in Aphasia – PALPA (50, 51, 56, 58, 59)[43] and the Boston Diagnostic Aphasia Examination (BDAE)[46] . We also used the Rivermead Behavioural Memory Test[44] in order to assess memory performance and the Wisconsin Card Sorting Test[45] for the assessment of shifting ability.

Participants

The participants were 10 patients with probable AD who participated in the Cognitive Neuropsychological Intervention (CNI) and 10 patients with probable AD as control group. All the participants were matched with respect to age, education and mental state (MMSE) [$F(1,19)= 0.001$ $p>0.05$, $F(1,19)=0.16$ $p>0.05$, $F(1,19)=3,2$ $p>0.05$ respectively]. All participants came from the city of Thessaloniki in Greece, and from the nearby rural areas. They were outpatients of the third Neurology Clinic of "G. Papanikolaou" hospital, all of them native Greek speakers, with no auditory or visual problems. They could read and recognize the visual items of the tasks and none of them was institutionalized or had alcoholic or drug abuse history. Their demographic characteristics are presented in Table 1. All participants and their caregivers were informed about the procedure and gave their consensus for the testing and intervention.

The Cognitive Neuropsychological Intervention Program

When the clinical and neuropsychological assessment was completed, the participants were given a naming test with colorful pictures of everyday life objects (200 items). The pictures involved objects from five categories (*food, tool, fruit, vegetable, animal).* Each picture presented items of the same category. The items ranged in familiarity from very familiar, familiar, to unfamiliar; they also differed in terms of ease of accessibility (following a pilot study with normal adults): easy, moderately difficult and difficult to access.

Design-Procedure

During the first phase of the intervention program the examiner recorded the non recognized or non named items without giving any semantic or phonological cues. In the second phase, the participants practiced on memory strategies. The aim was to help the person learn how to activate and reorganize the intact memory information that pertained to objects of the pictures or to relearn new information about the failed items. Thus the patients were asked to identify semantic relations (e.g., categorical or thematic) between the objects based on their existing knowledge of them. If they could not form some meaningful relation between the objects, the examiner gave semantic cues during the encoding in order to help the patient encode the information in a meaningful manner and incorporate the unknown object into the information that he/she retrieved from memory. In the third phase the patient practiced on each picture with the same or new relations between the objects, until *errorless learning (i.e., naming of all objects and the picture)* was achieved. This practice phase involved retrieval of information about the function, semantic relations, semantic associations, visual characteristics of the objects, as well as finding links between them. Patients were also asked to associate all of the objects with their lexical label.

There were three groups of sets of pictures in terms of ease of accessibility of the picture objects (the easy, the moderately difficult and the difficult one). Each session involved twenty eight pictures with four objects on each picture, that is 112 objects. It lasted 50-60 minutes. In the first month of the training, the learning of memory strategies was based on objects that had not been presented during the pretest naming condition. The aim was to help the patient find the best for him/her strategy for retrieving the object of each picture, as the material was organized. Among the best preferred by the patients strategies was to create a story that connects the objects of the picture, to find similarities or differences between the objects, to classify the objects according to a rule (color, size, shape, function). After the patients had found the appropriate strategy for the recall and naming the objects of each picture, they practiced on it until errorless learning was achieved. After that, the examiner tested the recall of the information and the

Table 1. Demographic characteristics of the sample.

Group	Age		Education		MMSE	
	Mean	SD	Mean	SD	Mean	SD
Control Group	75.3	3,6	11.3	4.8	22.5	2.4
Experimental Group	75.2	4	10	5.6	24.7	0.9
Mean	75.3	4	10.8	4.9	23.4	2.1

effectiveness of the memory strategies in recall by hinting at four objects per four pictures and asking the patient to name them bringing in mind the trained information about them. This procedure was repeated with all three groups of sets of pictures (easy, moderately difficult, difficult). If the patient could not find the correct name of the object, the examiner gave semantic cues that were used in the encoding phase and the patient tried again. If the patient failed again, the examiner gave a phonological cue (usually the first syllable of the lexical label) in order to facilitate phonological access. In the following sessions, the testing demands were increased, the examiner hinted at eight items per four pictures and asked the patient to name them. In this way the connections between semantic and phonological information were strengthened.

If the patients gave 80% correct answers in a group of set of pictures then they proceeded to the next group, which was more difficult. If the patients failed the 80% limit, they repeated the same group of pictures in the following session.

The duration of the intervention program was four months with three hour/ week sessions and home exercises for the rest of the days of the week. Home exercises aimed at rehearsing the relearned or the reorganized information about the objects; also the patient tried to find objects that belonged to the same superordinate or subordinate category with the same function or characteristics, as the objects in the rehearsal pictures. In this way generalization to non trained items was achieved. This was a kind of self-generation of category of objects activity[16].

In the last phase of practice we added to the material a set of cards that had the name of the objects in writing rather than in pictorial form. This was seemed necessary in order to help patients use semantic memory strategies and not only visual memory strategies. The training with cards followed the same pattern as the training with pictures. After 80% correct recall, the patient proceeded to the final phase, otherwise they practiced again. In the final phase we tested the naming of the failed pretest items.

A follow up after three and after six months of the completion of the intervention program was also implemented. At the three months follow up we retested only the naming of 200 items. But after the period of six months we retested the patients to all the neuropsychological tests of the pretest condition.

Results

We used t-test for independent groups in order to find out if there were differences between the experimental and control group in the naming ability in the pretest condition. We found that there wasn't any statistically significant difference in the pretest conditions between groups in the naming confrontation test [$t(2,18)=0.98$, $p>0.05$]. The repeated measures with the ANOVA 2x3 with the pretest scores, three and six months after the intervention scores as within subjects factors showed statistically significant difference between the groups and the testing:
$F(2,18)=14.7$, $p=0.005$,
$F(2,18)=13.6$, $p=0.006$,
$F(2,18)=13.3$, $p=0.006$, respectively.

This means that the intervention program helped the experimental group to improve its performance comparing to the control group to the three occasions and that this improvement lasted at least six months. Table 2 shows the mean scores and SD of the correct naming in the three testing occasions for both groups.

In order to test the hypothesis regarding the transfer of training to non trained abilities in the experimental group we used the t-test for matched groups between the pretest and retest scores. We found statistically significant differences in all the neuropsychological tests between the pretest and posttest scores. Table 3 presents the mean scores and SD on the neuropsychological tests before and after the intervention. The retesting took place six months after the completion of the intervention. The greatest differences were found in the immediate and delayed recall of the story subtest of the RBMT, in the verbal fluency ability regarding the animals (BDAE), to MMSE and CAMCOG scores, to BNT with the phonological cues, to the Wisconsin test, and the recognition of unknown faces of RBMT. This means that in the case of stories patients after six months of the intervention program used better strategies of encoding the information and recalling thus minimizing their omissions or mistakes. In the Animals task the experimental group used the trained strategies in order to

Table 2. Mean, SD of naming performance as a function of group and testing.

Group	Scorepretest		Scorepost3		Scorepost6	
	MO	SD	MO	SD	MO	SD
Control	104.2	11.6	116.8	37.1	113.7	37.3
Experimental	98.5	11.8	187.2	5.6	183.5	3.1

Scorepretest: scores in naming in the pretest conditions.
Scorepost3: scores in naming after 3 months from the intervention program.
Scorepost6: scores in naming after 6 months from the intervention program.

Table 3. Means and SD of the neuropsychological assessment of the experimental group.

Conditions	MMSE		CAMCOG		BNTphonol		ANIMALS		STORY[1]		Delayed STORY[1]		FACES RBTM		PPT		WISCONSIN[2]	
	MO	SD	MO	SD	MO	SD	MO	SD	MO	SD	MO	SD	MO	SD	MO	SD	MO	SD
pretest	24.7	.96	78.5	6.5	43.2	2.5	14.2	1.7	15.5	1.3	16.5	2.9	2	1.4	42.2	1.7	3	.5
posttest	27	.81	88.2	1.5	49.2	2.5	23.5	5.3	13	2.9	13.7	2.	4	.5	47.8	5.5	7	.96

Notes: 1: The score represents number of wrong answers/missions.
2: The score represents number of categories.

retrieve from memory existing information following a rule, i.e., to retain it in memory and not repeat it again. The better scores in the Faces subtest of RBMT suggest that besides verbal material, the use of strategies transferred to visual /facial material also. The participants also took advantage of the phonological cues in BNT and showed an improved shifting ability from one rule to another and from one category to another, as indicated by the Wisconsin test (table 3).

Conclusions

As we showed in the introduction there is a lasting debate regarding the effectiveness of cognitive support in AD patients. However our results are consistent with the findings of recent studies showing that cognitive intervention can have long term and generalization effects in the first stages of AD. The results showed that the patients achieved reorganization of intact and relearned information using memory strategies. The memory strategies helped the interconnecting of faded semantic information and the association between the meaning and the lexical labels of the items. They also transferred these strategies to the processing of other cognitive tasks as we noticed from the improvement of their scores in the memory and attention scales.

We found that patients in the first stages of AD can reorganize existing intact memory information by forming new associations and relations between items, learn new information, even maintain it at least for six months after the intervention program without feedback and without any notable decline in naming ability. However, for the intervention to be successful it is important for the patients to be motivated to take part in the program and the examiner to use items related to everyday life activities. In this way we can encourage transfer to actual life conditions. A friendly and supportive environment is also needed so that the patient feels comfortable and maintains effort through all the phases of the intervention program.

The question is if the patients and their relatives are aware of this change and if the training had effects on the patients everyday functionality and social skills. These are questions to be pursued in future research.

Table 4. T-test scores of the neuropsychological tests in pre and posttest conditions for the AD intervention group.

Test	Retest- 6 months	t-test	p=.010
MMSE1	MMSE2	t=-6	p=.004
CAMCOG1	CAMCOG2	t=-4.6	p=.010
BNTphonolog1	BNTphonolog2	t=-6.7	p=.003
Animals1	Animals2	t=6.1	p=.004
PPT1	PPT2	t=-3.3	p=.030
PPPTIME1	PPTTIME2	t=3.3	p=.029
Story1 RBMT	Story2 RBMT	t=3.8	p=.019
DelayedStory1	DelayedStory2	t=5	p=.007
RBMT	RBMT		
Faces1 RBMT	Faces2 RBMT	t=-6.3	p=.003
Wisconsin1	Wisconsin2	t=-5.3	p=.006

Unfortunately, information about the pretest of the neuropsychological tests is not available at this moment, because data collection is still in progress.

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Carotid Endarterectomy in Elderly Patients

Surgical Experience in a Hospital-Based Study

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Abstract

Background and purpose: ***According to the recent American Heart Association (AHA) Guidelines for Carotid Endarterectomy (CE), the benefit of carotid endarterectomy is highly dependent of surgical risk. Our purpose is to assess the level of our surgical team performance in carotid endarterectomy in a selected elderly population with asymptomatic and symptomatic carotid atherosclerotic disease.***
Method: ***The patients were eligible for study if they were >75 years old and presented a carotid bruit to the physical examination.The following risk factors were assessed: history of stroke/TIA, hypertension, coronary heart disease, diabetes mellitus, current\former cigarette smoking, hypercholesterolaemia. We screened 28 patients using carotid duplex ultrasound and/or angiography. A number of 18 patients with indication for CE were identified.***
Results: ***We performed CE in 14 patients. No deaths, nondisabling or disabling stroke occurred at 30 days. A local minor complication (subcutaneous haematoma) was registered. The patients are still involved in a comprehensive follow-up program (neurological, cardiological and psychiatrist evaluation).***

Introduction

Demographic data prove that we assist to a permanent ageing of population with a consistent increasing in elderly population across the world. One half of the total number of elderly are living today. They represented 14% of the population in 2000, but in 2020 they are estimate to range 22%. Among the sixty years old persons, one half have a minimum of 85 years live expectancy. This ageing trend is the most powerful in the developed countries, where the 85 years category has the most highly increasing rate[1].

Consequently, this segment of population will determine health care system and society to pay them more and more attention. The particularities of the elderly consist in functional deterioration, associate pathologies and polipharmacy. The cornerstone of the patology in aging is atherosclerosis, with different localization: cerebral, coronary or

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peripheral. In this context, we understand why we consider that the atherosclerotic carotid disease has to become one of the major themes of interest in a large team of specialities, from the general practitioner to cardiologist, vascular surgeon, neurologist and psychiatrist.

In this article we will try to assess the carotid endarterectomy (CE) in the elderly, from a vascular surgeon approach in a selected population, according to AHA Scientific Statement of Guidelines for Carotid Endarterectomy[2].

Clinical trials data

Three trials regarding the role of CE in the treatment of patients with symptomatic carotid stenosis are wellknown: The North American Symptomatic Carotid Endarterectomy Trial (NASCET). The European Carotid Surgery Trial (ECST), and Veteran Affair Clinical Studies Program 309.

In August 1991, the initial results from NASCET demonstrated the highly beneficial effect of CE in patients with high-grade carotid stenosis (70-99%) angiographically assessed; the benefit is durable[3].

In the report of surgical results in the 1415 patients who underwent CE in the surgical arm of the NASCET the overall rate of perioperative stroke and death was 6.5%; at 30 days - death 1.1%, disabling stroke 1.8%, nondisabling stroke 3.7%.The risk of perioperative wound complications was 9.3% and that of cranial nerve injuries was 1.8%[4].

There were identified five baseline variable as predictives for an increased surgical risk: hemispheric versus retinal transient ischemic attack (TIA) as the qualifying event, left-sided procedure, contralateral carotid occlusion, ipsilateral ischemic lesion on CT scan, and irregular or ulcerated ipsilateral plaque detected by angiography. One baseline variable was unexpectedly associated with a significant reduced risk of perioperative stroke and death: patients with a history of coronary artery disease (CAD) who had a prior cardiac procedure[4].

None of intraoperative variables examined (monitoring, intraluminal shunt, the choice of suture size, the form of anesthesia, the nature of closure of the arteriotomy) were correlated with a statistically significant increase or decrease in perioperative risk[4].

The results reported from the European Carotid Surgery Trial (ECST) – a NASCET-similar-size trial – are comparable.The authors concluded: in severe, symptomatic carotid stenosis (=70%), the benefit from CE is very significant and increases dramatically as the stenosis increases up to 95%.

A Multidisciplinary Consensus Statement from the American Heart Association concluded that CE is of proven benefit for symptomatic patients, including those with single or multiple TIAs or those who have suffered a mild stroke within a 6-months interval, who have stenosis of greater than 70% with a surgical risk of less than 6%[2].

The largest trial concerning the management of asymptomatic carotid stenosis (ACAS) enrolled patients with asymptomatic carotid stenosis >60%; the primary endpoints of this study were stroke ipsilateral to the carotid stenosis or death within 30 days of randomization. The risk for the primary outcome was 10.6% in the medical group versus 4.8% in the surgical one. The results demonstrated that CE provided a statistically significant benefit with an absolute risk reduction of 5.8% and a relative risk reduction of 55% in the risk of the primary endpoint of stroke within five years[6,7].

Personal experience

We developed a comprehensive program for detection, treatment and long term management of the patients with carotid stenosis.

Subjects and methods

We performed screening procedures in 108 patients addressed by different specialities physicians for carotid stenosis diagnosis. The patients >50 year old were eligible for the study in presence of a carotid bruit. From this group, we pay attention in this article to the subset of patients >75 years old. In the elderly category, 20 were aged 75-79 years, 6 were 80-84 years, and two 85-89 years. Analysing the baseline risk factors and comorbidities in the lot of the elderly patients, the results are: sex distribution – male 20 patients (71.4%); Doppler ultrasound carotid stenosis detected a significant carotid atherosclerotic disease (>50% stenosis) in 18 cases (64.3%); from this 12 patients have a high degree carotid stenosis (>70%).

Eleven patients (39.3%) presented a previous stroke or TIA at the time of screening procedures. Regarding the associated comorbidities, coronary artery disease was diagnosed in 17 cases representing 60.7% of the aging population, arterial hypertension in 14 patients (50%) and diabetes mellitus in 7 cases (25%). Eight of the patients (28.6%) were current or former cigarette smoker (Table 1).

Patients in the elderly category had almost a similar distribution of risk factors comparing to the younger patients regarding diabetes, hypercholesterolaemia, hypertension. Cigarette smoking declined substantially with increasing age. A dramatically increasing of the coronary artery disease was registered (60.7% vs. 43.7%) and also the history for stroke/TIA (39.3% vs. 17.5%). In addition, in the oldest age category a smaller proportion of patients had a transient ischemic attack than stroke.

Eighteen patients of this category had a significant carotid stenosis (64.3%). A preoperative carotid angiography was performed in 16 patients in the Department of Invasive Cardiology of Timișoara

Table 1. Baseline characteristics in the elderly patients(≥75 years) subgroup.

Characteristics	n=28	%
Male	20	71.4
Female	8	28.6
Doppler ultrasound carotid stenosis		
<50%	10	35.7
50-69%	6	21.4
≥ 70%	12	42.8
Previous TIAs and stroke	11	39.3
CAD	17	60.7
Hypercholesterolaemia	12	42.4
Hypertension	14	50
Diabetes mellitus	7	25
Cigarette smoking	8	28.6

Institute of Cardiovascular Medicine (Figure 1).

Among the high degree carotid stenosis subset of patients (>70%), nine were with history of stroke /TIA. Two asymptomatic patients (50-70% carotid stenosis) refused the surgical treatment and two patients with 50-70% carotid stenosis with symptomatic CAD were addressed to the Cardiovascular Surgery Clinic of Timișoara Institute of Cardiovascular Medicine for coronary artery by-pass grafting for myocardial revascularization and CE.

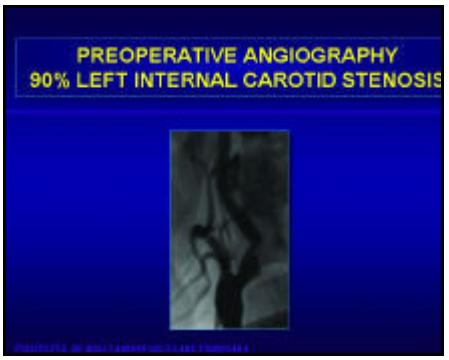

Figure 1. Preoperative angiography in a 76 years old male patient. 90% stenosis of left internal carotid artery

Standard CE was performed in 14 patients without using patch or intraoperative shunt. Data regarding the type of intervention according to the history of stroke and degree of carotid stenosis are summarized in Table 2.

Table 2. Type of intervention according to degree of carotid stenosis and history of stroke/TIA.

	N	Type of intervention
Previous stroke/TIA	11	CE
50-70% carotid stenosis	9	
>70% carotid stenosis	2	
Asymptomatic		
50-70% carotid stenosis	2	CABG +CE
	2	Refused intervention
>70% carotid stenosis	3	CE

All interventions were performed in conditions of general anaesthesia, continous vital signs monitoring and intraoperative heparin sodium administration. The average period of clamping was 8 minutes. We consider the most important intraoperative time the carefully single plan removing of the atherosclerotic plaque in order to avoid restant flaps and shelves. Primary closure of the artery wound with Prolene 5.0 in continous suture was preferred by the surgical team. The unidirectional suture was followed by a systematically irrigation of proximal and then distal branches with heparin saline solution. According to the general surgical rules a meticulous inspection of sutures and a appropriate hemostasis were done. To avoid a postoperative haematoma we positioned a rubber drainage in vascular logia through the inferior pole of the incision (Figures 2-6).

Adverse outcomes included death, postoperative strokes, and wound complications.

A comprehensive step-by-step follow-up included: complexe monitoring (ICU) day one and two postoperative, local wound evolution daily (day 1-7), neurological assessment – day 1, 7 and 30 and every 6 months after, cardiologic assessment day 1, 7 and every 6 months, Doppler ultrasound every 6 months, neuropsychiatric poststroke follow-up of the patients, in order to evaluate poststroke dementia–day 30 and every 6 months. The period of follow-up is 2 years (Table 3).

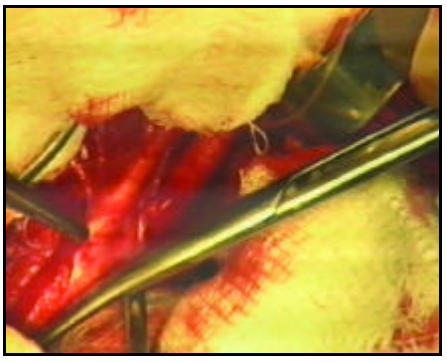

Figure 2. Intraoperative aspect :exposure of the carotid bifurcation.

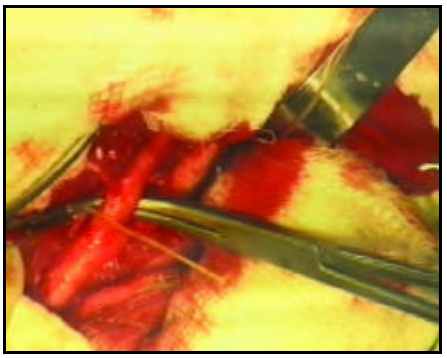

Figure 3. Before clamping: vessel loop across the internal carotid artery, external carotid artery and the common carotid artery were placed in sequence.

Figure 4. Potts scissors are used to elongate the incision proximally and distally across the area selected for endarterectomy.

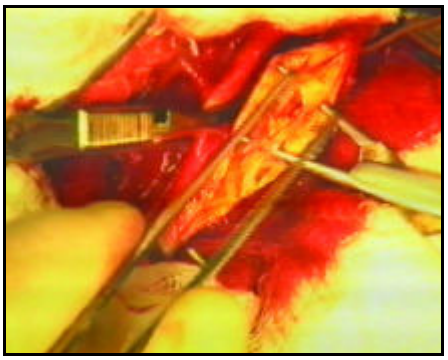

Figure 5. Carefully dissection of the atheromatous plaque. Single plan removing of the plaque. Delicate feathering of the endarterectomy. No flap or shelf can be tolerated.

Figure 6. Primary closure of the artery wound with 50 polypropylene in continous suture.

Results

Short term

No deaths, non disabling or disabling strokes were registered at 30 days after interventions.

One local minor complication represented by a subcutaneous haematoma occurred day one. It was resolved by local wash and drainage.

Long term

The patient entered into a long term follow-up program; the trial is still ontgoing. Periodical neurological assessment at every 6 months showed no additional strokes for 10 patients until this date; 4 of the patients are in the first postoperative 6 months.

Discussion

The benefit of CE in symptomatic patients with high-grade stenosis has been established in the North American Symptomatic Carotid Artery Trial and ECST. Furthermore, CE has been reported to be beneficial also in asymptomatic patients in the ACAS clinical trial[3-6,8].

Available data from literature showed that among surgically treated patients, those aged 75 years or older had a lower risk of ipsilateral stroke whatever the degree of internal carotid artery stenosis (Kaplan-Meier risks of ipsilateral ischaemic stroke at 2 years according to age and degree of stenosis in medical and surgical study groups). The elderly patients had the highest risk for stroke with medical treatment. Therefore, the absolute risk reduction by CE in these cases is the greatest: 28.9%, P<0.0001. There is enough to performe CE in 3 patients to prevent an ipsilateral ischaemic stroke at 2 years. The aggregate risk of ipsilateral ischaemic stroke in the surgical group at 2 years was 7.6% among the patients >75 years. In NASCET, the lower risk of ipsilateral ischaemic stroke at two years in the surgical group for the oldest age-group was due to a lower rate of perioperative stroke and death in this age category[9,10,11]. These three important studies, along with consensus statement from experienced clinicians, have been the principal sources for determining the appropriate indication for CE[2,12]. It is not clear that the results from these trials can be extrapolated to the community and nontrial conditions[8].

At the present time, the results in our study are encouraging. Actually, the small number of patients in this category is still not appropriate to release final results regarding the risk reduction and benefit of CE on long term of our surgical team. New patients have to be enrolled into the study in order to better assess our own surgical performance level and the appropriate therapeutic management of this high risk patients.

Conclusion

These data represent the partial results in a hospital-based study among a selected population for carotid atherosclerotic disease. This project raised as a necessity in order to assess the real level of own surgical team performance in CE, according to the standard requirements of the centres involved in large trials like NASCET or ECST. Lack of data regarding this procedures in East European Countries and poor information about the stroke epidemiology and risk factors of perioperative stroke in elderly were available in our population[12].

The identification of high-risk patients based on a pertinent risk factor analysis[13] in collaboration with the cardiologist and neurologist offers an important opportunity to improve the surgical outcome of this procedure.

Table 3. Timetable of follow-up procedures in elderly with CE.

PROCEDURE	DAY 1	DAY 7	DAY 30	EVERY 6 MONTHS
ICU monitoring	* and day 2			
Wound	* daily during 1-st week			
Neurological	*	*	*	*
Cardiological	*	*		*
Psychiatric			*	*
Carotid Doppler Ultrasound				*

Different authors (Brook et al, Karp et al) concluded that "appropiateness of care cannot be closely predicted from many easily determined characteristics of patients, physicians, or hospitals. Thus for the present, if appropiateness is to be improved, it will have to be assessed directly at the level of each patient, hospital and physician."[8]

We also consider as a promising research theme the evolution of poststroke dementia in these patients[14].

An interdisciplinary preoperative and postoperative evaluation will improve the surgical risk stratification, the appropriate selection of therapy, and the identification of the high-beneficial category of patients.

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Tumor of Cerebral Posterior Fossa Associated with other Intracranial Lesions

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Case presentation

The patient, 66 years old, female, whose optical neuritis diagnosis was established 17 years ago, and during the last 15 years she manifested disorder in walking and diplopia episodes quasitotally improved with conservative treatment, was hospitalized in our clinic because of a facial asymmetry and a left hemianopsy relative suddenly installed 3 week before the date of hospitalization.

The MRI cerebral exam using the contrast substance showed:

- a tumor of cerebral posterior fossa of 2/3 cm placed under the tentorium with a constrast enhacement which touches the transverse sinus with the aspect of meningioma.
- another tumor with small dimenssions, infracentimeters, with important contrast enhacement placed “in mirror” position comparing to the first, supratentorial, possibly meningioma, too.
- multiple vascular – degenerative lesions situated periventricular, with high signal intensity in T2.
- 2 subcutaneous lesions with tumoral aspect, disposed to the convexity of the skull with contrast enhacement to the margin.
- A lesion with a high signal intensity in T1 and T2 and a marginal low signal intensity without contrast enhacement, suggesting an intraparenchimatous bleeding, right occipital placed in connection with the occipital horn of lateral ventricle, without an important perilesional edema.

The patient presented 4 types of lesion: two benign tumors (possible meningiomas), situated supra and infratentorial, a lesion occipital right placed - haematoma, multiple subcutaneous lesions with tumoral aspect, and multiple vascular degenerative lesions periventricular disposed.

The general and neurological exams of the patient has emphasized a good general condition without locomotory or sensitive disfunction, excepting a facial left asymmetry and left homonymous hemianopsia, symptoms which are surely given by the intracerebral right occipital haematoma.

In case of these patients with benign tumors, asymptomatically, especially if they are associated with other intracerebral lesion, the recommended treatment is the conservative for associated lesions and the clinical and neuro-imagistic periodical exams every 3-6 months for the tumoral lesion.

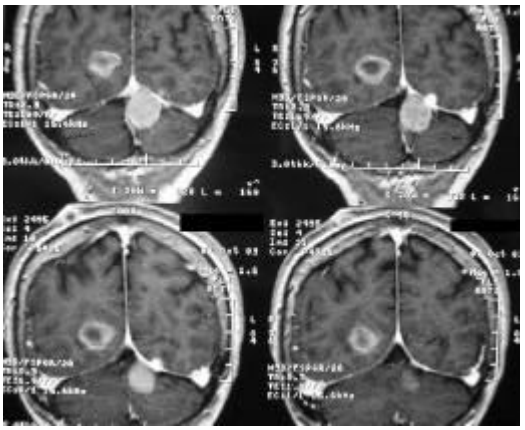

Figure 1. Paramagnetic enhancing coronal T1w FSE

In the long run, if it is noticed that tumoral lesion is stabilized from the dimensions point of view, the surgical intervention is not necessary. If the tumoral lesion grows and becomes symptomatic, the surgical sollution is recommended.

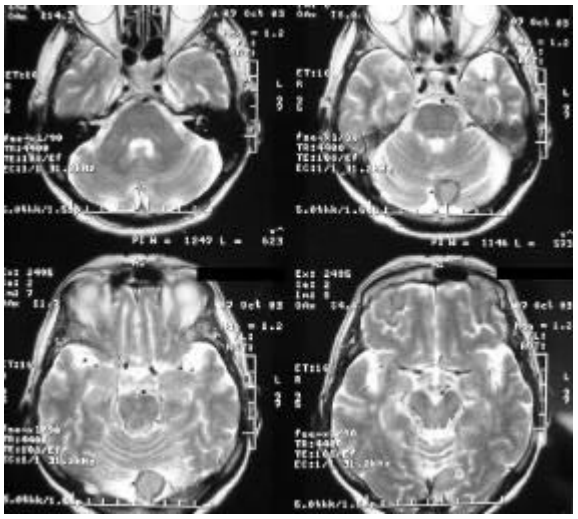

Figure 2. Axial T2w FSE

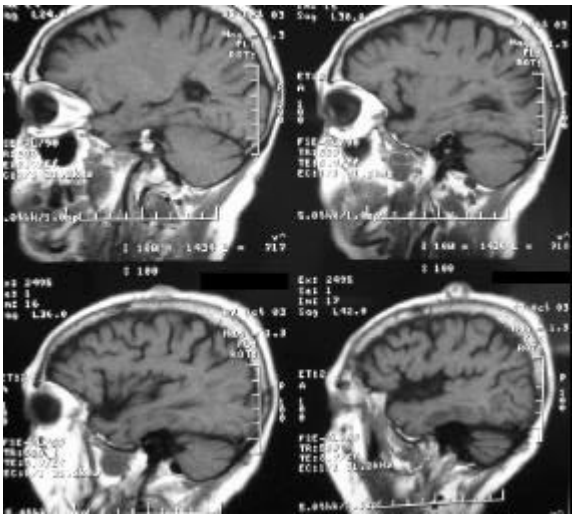

Figure 3. Sagital T1w FSE

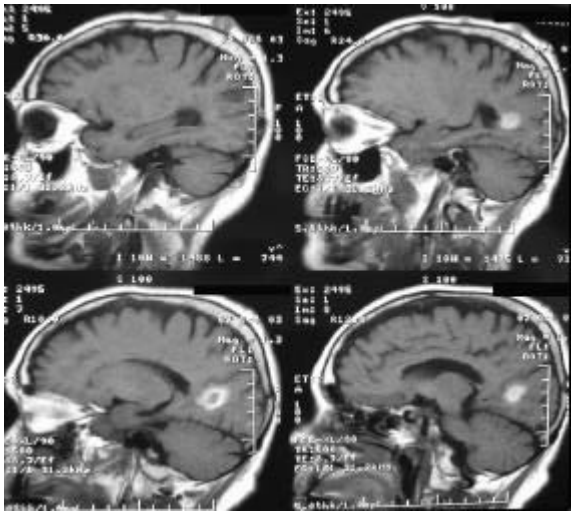

Figure 4. Sagital T1w FSE

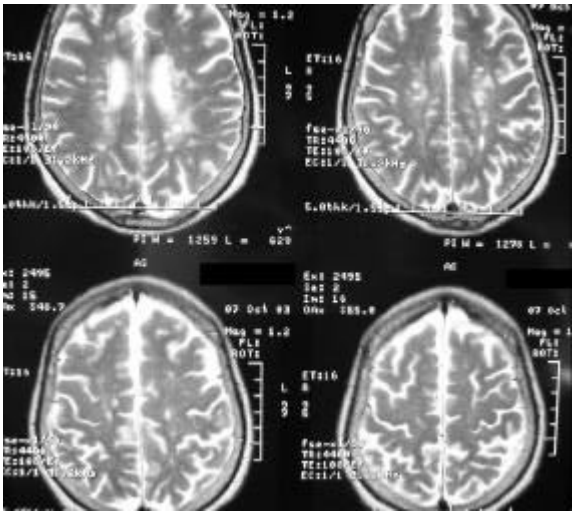

Figure 5. Axial T2w FSE

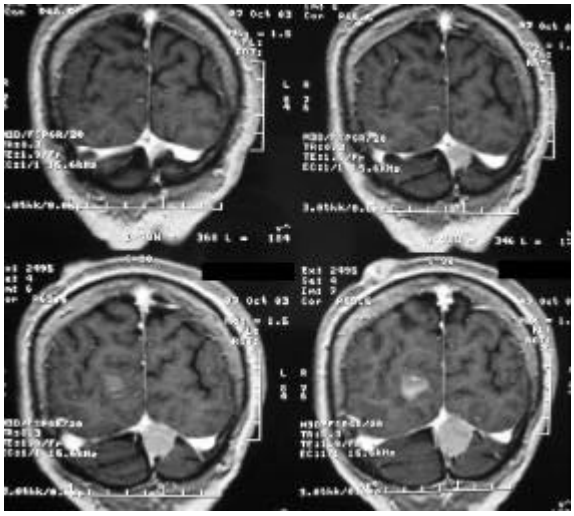

Figure 6. Paramagnetic enhancing coronal T1w FSE

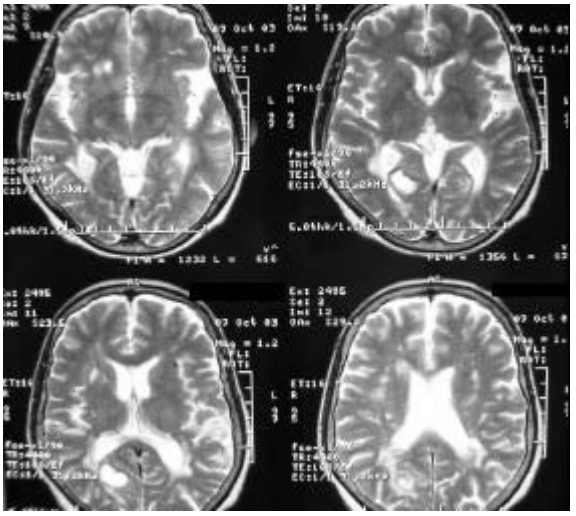

Figure 7. Axial T2w FSE

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Plenary Speakers

Join your colleagues in Philadelphia and take advantage of this exciting opportunity to learn from leading experts in Alzheimer research. The Scientific Program Committee has selected 15 seminal plenary topics and invited the following not-to-be missed plenary speakers.

Sunday, July 18, 2004

Epidemiology of Alzheimer's Disease:
Lessons from Cardiovascular Studies
Lenore Launer, PhD
National Institute on Aging, Bethesda, Maryland U.S.A.

Genetics of Alzheimer's Disease
Gerald Schellenberg, PhD
University of Washington, Seattle, Washington, U.S.A.

ApoE and Alyheimer's Disease: A 10-Year Update
Karl Weisgraber, PhD
University of California, San Francisco, California, U.S.A.

Monday, July 19, 2004

Oxidative Mechanisms, Inflammation,
and Alzheimer's Disease Pathogenesis
Flint Beal, MD
Cornell University, Ithaca, New Tork, U.S.A.

Biological Function of APP
Edward Koo, MD
University of California, La Jolla, California, U.S.A.

Tau and Tauopathies
Eva-Maria Mandelkow, MD, PhD
Max Planck Institute, Hamburg, Germany

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- Genetics and genetic testing
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- Neuropathology and imaging
- Molecular and cellular biology
- In vivo and vitro models system
- Established and experimental therapeutics
- Brain-behaviorals relationships
- Evidence-based patient management and social-behavioral research
- Ethical issues in dementia care

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Sunday, July 18, 2004

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For More Information
Independence Visitor Center
1.800.537.7676
www.gpphila.com

Alzheimer's Disease and Related Disorders

Tuesday, July 20, 2004

Behavioral and Psychological Symptoms of Dementia: Nature and Treatment
Constantine Lyketsos, MD
Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.

Pharmacological and Non-Pharmacological Treatments in Alzheimer's Disease
Rachelle Doody, MD, PhD
Baylor College of Medicine, Houston, Texas, U.S.A.

Therapeutic Strategies for Alzheimer's Disease
Steve Paul, MD
Eli Lilly and Company, Indianapolis, India, U.S.A.

Wednesday, July 21, 2004

Cellular Models of Alzheimer's Disease
Nikolaos Robakis, Phd
Mount Sinai School of Medicine, New York, U.S.A.

Animal Models of Alzheimer's Disease
Karen Hsiao Ashe, PhD
University of Minnesota, Minneapolis, Minnesota, U.S.A.

Biology of Gamma Secretase
Takeshi Iwatsubo, MD
University of Tokyo, Tokyo,Japan

Thursday, July 22, 2004

Molecular Pathology/Histopathology of Alzheimer's Disease
John Q. Trojanowski, MD, PhD
University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.

Imaging in Prediction and Longitudinal Assessment of Alzheimer's Disease
Clifford R. Jack, MD
Mayo Clinic, Rochester, Minnesota, U.S.A.

Biomarkers in Alzheimer's Diasease
Kaj Blennow, MD, PhD
University of Goteborg, Molndal, Sweden

Important Details

Abstract Submission

Beginning in Septembrie 2003, information about submitting abstracts will be available on-line. Abstract will be solicited for both oral and poster presentation on all aspects of Alzheimer's disease and related disorders, including epidemiology, genetics, molecular and cellular biology, pathology, neuroimaging, experimental therapeutics and evidence-based patient management and social-behavioral research.

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On-line submission: November 1, 2003 to February 2, 2004. Submit abstracts electronically at: *www.alz.org/internationalconference*
Notification date: March 2004
Registration book available: January 2004

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Exhibitors will include pharmaceutical companies displaying information about current and investigational therapies; print and electronic publishers of scientific resources; and governmental and non-governmental agencies. The Alzheimer's Association invites you to generate new and untapped leads, meet your customers face-to-face, and establish your brand to a qualified scientific market of more than 5,000 Alzheimer researchers.

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Exemples:

5. Bierer L.M., Hof P.R., Purohit D.P., Carlin L., Schmeidler J., Davis K.L., Perl D.P., Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol* 1995; 52: 81-8. [for journal articles]
6. Benton A., Tranel D., Visuoperceptual, Visuospatial, and Visuoconstructive Disorders. In: Heilman K.M. and Valenstein E., eds., *Clinical Neuropsychology*. Oxford University Press, 1993: 195-212 [for edited books]
7. Luria A., *The Working Brain*. New York: Basic Books, 1973.[for monographs]

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