campal index was only 0.6 times the mean, while that of ADAS-Cog was 1.7 times. Since image processing is a uniform mechanical task, variation of the imaging biomarker should be small. Sensitive biomarkers which reliably and objectively reflect changes in lesions, even though the effect size is small, are expected to be used analogously to commonly used laboratory test indices for evaluation of the disease severity in clinical practice such as C-reactive protein in inflammatory diseases, serum transaminase levels in liver diseases as well as serum creatinine kinase levels in muscular diseases. Thirdly, we need biomarkers that support evaluation of therapeutic effects. Several classes of amyloid-reducing drugs such as γ-secretase inhibitors (De Strooper et al. 2010) and amyloid immunization therapy (Tabira 2010) might become available in the near future. For the development of these therapeutic drugs, development of methodology to objectively access "decrease or removal of amyloid" is necessary. For example, when the brain amyloid level is reduced by a novel treatment, the biomarker levels are expected to return closer to normal range. Ideal biomarkers may also provide important information regarding the timing of treatment initiation, discontinuation and changing of drug treatment. However, it may be unlikely that a single biomarker meets all conditions described above, and it may be more realistic to prepare a combination or panel of several different biomarkers.

Since therapy is likely to be most effective at or before symptom onset, early or pre-symptomatic detection of AD is highly desirable before neurodegeneration becomes obvious. Thus, there is a great need for blood and CSF biomarkers that substantially aid tracking disease progression of AD and eventually promoting prevention strategy. As reviewed elsewhere (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association & NIAWG 1998; Frank et al. 2003), ideal AD biomarkers should detect a fundamental feature of AD neuropathology, be validated in autopsy confirmed cases, have a diagnostic sensitivity > 80% for detecting AD and a specificity of > 80% for distinguishing AD from other dementias. Moreover, assays using AD biomarkers should be reliable, reproducible, non-invasive, simple to perform and inexpensive. Further, validation of AD biomarkers requires confirmation by at least 2 independent studies from qualified investigators published in peer-reviewed journals. Tau and Aβ are major components of the two neuropathological hallmarks of AD (tangles and plaques respectively), and they are the most intensively studied candidate AD biomarkers where they are best studied in cerebrospinal fluid (CSF) using extensively characterized ELISAs (Arai et al. 1995; Arai et al. 1997; Arai et al. 1998; Tomita et al. 2007). A recent examination of > 100 subjects with autopsy-confirmed diagnoses reached a conclusion that elevated CSF tau levels are associated with the presence of AD pathology and CSF Aβ42 levels are decreased in AD (Clark et al. 2003). Currently, it is widely accepted that biomarkers of brain amyloid burden are reductions in CSF Aβ42 and increased amyloid PET tracer retention (Fagan et al. 2006; Jack et al. 2010). As shown in Fig. 2, after a lag period, which varies from patient to patient, neuronal dysfunction and neurodegeneration become the dominant pathological processes. Biomarkers of neuronal injury and neurodegeneration are increased CSF tau and structural MRI measures of cerebral atrophy (Arai et al. 1995). Neurodegeneration is accompanied by synaptic dysfunction, which is indicated by decreased FDG-PET (Jack et al. 2010).

Development and clinical applications of amyloid imaging

Amyloid imaging is currently considered to be the most promising candidate biomarker since it meets many possible conditions of an ideal biomarker as described above. The most difficult hurdle for clinical application of this technology is to find a probe with following excellent characteristics: 1) it should selectively bind to Aβ aggregates with β-sheet-structure; 2) it should readily penetrate the blood-brain barrier (BBB) while being rapidly cleared off from the brain in the absence of the target; 3) the labeled form should not lose the characteristics of the mother compound. In our experience, enhancing one of several necessary characteristics causes loss in another, requiring extensive adjustment.

Although brain Aβ deposits are still well beyond the resolution of conventional neuroimaging techniques such as MRI, the density of these deposits in the brain tissue can be visualized through specific radiotracer and positron emission tomography (PET). The first compound to emerge as an amyloid-imaging agent was Chrysamine-G (Klunk et al. 1995). This compound shows similar binding characteristics to Congo-red, but unfortunately, due to its limited BBB permeability, there was no use as a clinical PET tracer. A marked progression in the development of amyloid-imaging tracers was made by the development of 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl) amino]-2-naphthyl}ethylidene) malononitrile ([^{18}F]FDDNP) (Agdeppa et al. 2001). This compound is highly lipophilic and can easily cross BBB, and has been used in human PET studies (Shoghi-Jadid et al. 2002; Small et al. 2006; Barrio et al. 2008). However, this agent has some limitations in its practical use due to its low signal-to-background ratio (Tolboom et al. 2009). Currently, the most successful amyloid-binding agent is a thioflavin-T derivative, N-methyl-[^{11}C] 2-(4′-methylaminophenyl)-6-hydroxybenzothiazol ([^{11}C]PIB) which has been shown to possess a high affinity for Aβ fibrils (Klunk et al. 2003; Mathis et al. 2003; Klunk et al. 2004). An autoradiographic study using AD brain sections revealed that [^{11}C]PIB, in addition to binding to the classical fibrillar Aβ plaques, also binds to a range of Aβ containing lesions including diffuse plaques and cerebrovascular amyloid angiopathy (Lockhart et al. 2007). In vitro binding studies indicated that PIB preferentially binds to Aβ1-42 fibrils with high affinity (Klunk et al. 2003) with a negligible binding to α-synuclein and tau (Lockhart et al. 2007; Fodero-

Tavoletti et al. 2007). The [^{11}C]PIB retention in the neocortical areas is correlated with the Aβ plaque load (Bacskai et al. 2007; Ikonomovic et al. 2008) with an inverse relation to CSF Aβ42 levels (Fagan et al. 2006). The frequency of cognitively normal individuals with positive PIB binding rose in an age-dependent manner from 0% at ages 45-49 years to 30.3% at ages 80-89 years. (Rowe et al. 2007; Morris et al. 2010). Further, CSF tau and phospho-tau$_{181}$ increased with cortical PIB binding in cognitively normal individuals (Fagan et al. 2009). However, there is currently no evidence of how frequently PIB-positive normal individuals will convert to develop dementia or how long is the interval between the detection of significant Aβ burdens and the onset of dementia. Longitudinal amyloid imaging studies are needed to demonstrate the reality of amyloid hypothesis via looking at relation between amyloid deposition and temporal AD progression.

Benzoxazole derivatives are also promising alternatives as amyloid-imaging probes (Okamura et al. 2004). A PET study using the ^{11}C-labeled benzoxazole derivative 2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy) benzoxazole (BF-227) demonstrated significantly higher retention of this tracer in cerebral cortices of AD patients compared to the majority of healthy elderly subjects (Kudo et al. 2007). The retention of this tracer in cerebral cortices of mild cognitive impairment patients was intermediate between AD and healthy normal subjects (Waragai et al. 2009; Furukawa et al. 2010). A voxel-by-voxel analysis demonstrated a higher retention of [^{11}C]BF-227 in the posterior association cortex of AD patients. The pattern of this distribution corresponds well with the distribution of neuritic plaques in postmortem AD brains (Okamura et al. 2009). These findings suggest [^{11}C]BF-227 may be distinct from [^{11}C]PIB in detecting different populations of amyloid deposits. In addition, glucose metabolism demonstrated by FDG-PET was negatively correlated with that of BF-227, suggesting that extracellular amyloid surrounds synapses and impairs neuronal function (Furukawa et al. 2010). In my personal view, a highly expected value of amyloid imaging may be its capability to monitor treatment effects in PIB or BF-227 positive normal individuals who have received amyloid-reducing therapies (Rinne et al. 2010). The [^{11}C]-labeled form has a short half-life (20.4 minutes) and its synthesis requires a facility capable of radioisotope synthesis using a cyclotron, whereas the [^{18}F] -labeled form has a longer half-life (109.7 minutes), which may be amenable for delivery to various sites. Therefore, the [^{18}F]-labeled compounds, for example, [^{18}F]AV-45 will probably be a standardized agent for future clinical uses (Personal communication from Skovronsky D).

Future prospects of the Japanese ADNI

Development of curative molecular targeting therapy for AD has rapidly progressed centering mainly in work done by U.S. pharmaceutical companies. Clinical trials of symptomatic treatments currently on the market could be completed within about 6 months, but planned disease-modifying drugs to delay progression of AD may require trial durations of at least one year or longer to confirm sufficient drug effect. Development of a surrogate biomarker which reflects the pathology of the disease and monitors its progression may be desperately needed for conducting long-term clinical trials. Based on this consideration, an observational clinical study called "The Alzheimer's Disease Neuroimaging Initiative (ADNI)", was proposed and initiated in the U.S.A. in 2005 (Mueller et al. 2005; http://www.adni-info.org/ ; http://www.loni.ucla.edu/ADNI/). ADNI is a non-randomized long-term observational study undertaken in the U.S.A., Europe, Australia, and Japan using an identical protocol in each participant nation. Japanese ADNI (J-ADNI) is planning to follow 300 patients with MCI for 3 years, 150 patients with early AD for 2 years, and the other 150 normal subjects for 3 years in a cooperative study of a total of 38 facilities nationwide with sufficient experience in the management of dementia (http://www.j-adni.org/). The principle investigator is Professor Takeshi Iwatsubo at University of Tokyo. The study objectives are: 1) to establish methodology that will determine standard values related to long-term changes in image data, such as MRI and PET, in AD and MCI patients and normal elderly persons; 2) to simultaneously collect clinical indices, psychological tests, and blood/cerebrospinal fluid biomarkers to demonstrate the validity of image surrogate markers, and 3) to establish the optimum method to monitor therapeutic effects of curative drugs (disease-modifying drugs) for AD, for which analyses of the following observation items are prioritized: 1) Rate of conversion from MCI to AD, 2) rates of whole brain and hippocampus volume changes via MRI, 3) rates of change in blood and cerebrospinal fluid biomarkers, and 4) rate of change in glucose metabolism on FDG-PET. In addition, baseline amyloid PET scans are given to subjects who agreed it in J-ADNI. We hope that J-ADNI project promotes long-delayed improvements of Japanese infrastructure of medical care system for dementia. It is inadvisable for Japanese medical society to ignore that in the U.S.A. a paradigm shift in AD from 'cognitive measures-based to biomarker-based' has begun after deliberation and discussion on subjects such as clinical trial efficiency and cost reduction. Many different curative drugs are under development by pharmaceutical manufacturers, and global clinical trials of these new drugs are ongoing.

In J-ADNI, firstly, several of Japanese version of the cognitive test batteries were revised by Sugishita M. et al. to normalize the relative difficulty and to enhance maximum compatibility of the test with World Wide ADNI and later for global clinical trials of new drugs. The first patient was successfully enrolled at the National Center of Neurology and Psychiatry in July 2008. More than 330 patients have already been enrolled as of March 10, 2010. The consent rate to FDG-PET, amyloid PET, and sampling of cerebrospinal fluid was obtained from 80, 44, and 40% of the participants, respectively. We will attempt to increase the

number of patients enrolled and the rate of consent to biomarker sampling, aiming at a great success of J-ADNI and World Wide ADNI together.

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総説

私の認知症研究

荒井啓行

会長講演としての「私の認知症研究」は，謂わば「What have you done in dementia research ?」という疑問に答えるためのものである．私自身が独自の考えで立ち上げ・遂行してきた研究は，以下の3点であり，それ以上でもそれ以下でもないと思う．

1) アルツハイマー病バイオマーカーとしての脳脊髄液タウ
2) 漢方方剤「抑肝散」による認知症の問題行動・精神症状軽減効果
3) アミロイド分子イメージング（工藤幸司教授との共同研究）

研究者には誰でも将来の方向性を決めなければならない時がある．私の場合それは1980年代の後半に訪れた．認知症研究にとって，1980年代の後半とはどんな時代であったであろうか？ 1984年，米国カリフォルニア大学サンディエゴ校のGlennerらは，アルツハイマー病脳の髄膜血管からペプチド断片を抽出し，そのN末端側28アミノ酸配列を決定した．これまで知られていない新しい構造であった．今日のアミロイドβ蛋白（Aβ）である．同じ構造のペプチドが老人斑からも検出された．このアミノ酸配列をもとにAβ前駆体蛋白（APP）をコードする遺伝子配列が決定されたのが1987-88年である．APP遺伝子は第21番染色体上にあり，APPは細胞外ドメインが長く細胞膜を1回貫通する膜蛋白構造をとるものであった．スプライシングにより，3つのアイソフォームが存在することもわかった．一方，Iharaらは神経原線維変化の主要構成要素は微小管関連蛋白タウであろうところまで追い込んでいたが，Tanglesを形成した神経原線維変化は如何なる可溶化剤にも溶けずそれ以上の蛋白化学的分析を困難にしていた．また，Moriらがユビキチンをアルツハイマー病研究に登場させたのもこの頃である．これらの研究はアルツハイマー病が異常蛋白の不溶化による蓄積を出発点とする疾患であることを強く示唆するものであり，大いに私の興味を引くところとなった．と言うのも，学生時代から生化学に興味があり大学院時代も多少は基礎研究を経験していたからである．「ミトコンドリアに電子が流れる際，電子が（虚血で損傷された）電子伝達系から逸れて酸素の不完全還元が起こるか，言い換えれば活性酸素が発生するかを証明せよ」が，当時の指導教官で

My contribution to dementia research
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図 1. 左上：ペンシルベニア大学の John Trojanowski 氏, 右上：同大学の Virginia Lee 氏,
左下：初夏のペンシルベニア大学構内, 右下：1990 年頃の研究室風景

あった東北大学神経内科教授の小暮久也氏から与えられたテーマであった．活性酸素研究で活躍していた群馬大学の中野稔教授の教えも受けながら研究に取り組んだ．しかし，化学発光分析という当時としては新しい方法を導入しても簡単に証明できるものではなかった．博士論文も不十分なもので，3 流の英文誌に辛うじて掲載されたに過ぎなかった．しかし, 分子から理解する医学に興味を持った．そこへ, アミロイド, タウ, ユビキチンなどとアルツハイマー病が蓄積分子から理解されそうな時代が到来したのである．当時, 日本でのアルツハイマー病研究者は, 西村健，石井毅，平井俊策，前出の井原康夫，森啓など数える程しかおらず，診療面では精神科病棟の一部で重症となった認知症患者を入院させていた時代であり，国民の認知度も極めて低かった．そのような中で，1988 年米国ペンシルベニア大学神経病理学部門の John Trojanowski/Virginia Lee 両教授の門を叩くこととなった（図 1）．John Trojanowski 氏は神経病理学者，Virginia Lee 氏は生化学者で，2 人ともアルツハイマー病研究領域を走り始めたばかりの新進気鋭の研究者であった．脳虚血に関する 2, 3 の論文のみで，アルツハイマー病研究経験が全くない私をよくぞ雇用していただけたと今でもこの 2 人には感謝に堪えない．このあたりの時期が私の人生の一大転換期でありまた決定期でもあった．米国の若い大学院学生達が眼の色を変えてアルツハイマー病研究に取り組んでいる姿は極めて印象的であった．なぜ？ その答えはその後の米国でのアルツハイマー病研究に対する研究助成金の拡大を見れば一目瞭然である．米国は認知症患者数の増大を National Crisis とまで呼んでその制圧に真剣に向き合ってきたのである．

まず私が取り組んだのは，APP のアミノ酸配列に基づいてペプチドを合成しそれをウサギに注射して抗体を得ることであった．Up107 という抗体が中でも優れものであった．初期の老人斑も見事に染色した．「砂をさらっと撒き散らしたような不思議な Aβ 沈着」と思っていたところ，海の向こうの日本から Diffuse Plaques という名称で初期 Aβ 沈着が報告されていることを知った．群馬大学山口晴保先生の業績である．その後，Up107 で正常剖検脳を免疫染色してみると，Up107 陽性の老人斑が多数見られることがわかったが，そのほとんどは Diffuse Plaques であり，変性神経突起を伴っていないもの

であった．一方，アルツハイマー病剖検脳を Up107 で免疫染色してみると，Diffuse Plaques ばかりでなく変性神経突起やコアを持った多様な形態の老人斑がみられた．変性神経突起はタウや APP，ユビキチン陽性であった．この違いをいかに説明するのか？　ここに時間軸というものを据えれば，今日のアミロイド仮説の原形のようなものが見えてくるが，当時の私にはそこまで考えが及ばなかった．逆に，私は「Aβ は悪者ではなく，アルツハイマー病では Aβ よりもタウ蓄積の方が病的意義が大きいのではないか」と，その後の Hardy らによるアミロイド仮説の提唱など意に介さず，Tauist への道を自然に選んでしまったようだ．MGH の Hyman らは，記憶回路の一部である海馬 - 嗅内皮質系では神経原線維変化の方が優位であり，神経原線維変化は神経細胞死の程度と相関するが老人斑は相関しないことを報告しているが，私も同じような感覚であった．

その後，1991 年に Virginia Lee らにより可溶性 PHF 由来のタウの一次構造が確認され，神経原線維変化の基本骨格は何かという長年の議論に決着が付くことになった．今日でこそ脳脊髄液バイオマーカーは ADNI でもバイオマーカー研究のコア部分を占めているが，脳脊髄液からバイオマーカー開発を試みるという発想そのものは実はそう簡単に研究者仲間に受容されるものではなかった．脳脊髄液を採取することへの否定的反応も強かった．「PHF 形成の初期段階は可溶化したままのタウである．であるならば，脳脊髄液から（リン酸化）タウ蛋白を検出できるのではないか」という可能性を考え続けた．1991 年春に帰国することになったが，帰国直前の研究室で John Trojanowski と「脳脊髄液を収集する」ことを密かに話し合った．しかし，着想から実施に至るまでに 3 年を要した．最後に私の背中をポンと押してくれたのは，東北大学神経内科時代にお世話になった当時助教授であった高瀬貞夫氏であった．高瀬貞夫氏は多発性硬化症の脳脊髄液を研究材料としていた．いつも楽しそうに脳脊髄液を研究していた．脳脊髄液は研究ネタの宝庫かも知れないと思った．「ニューロンとニューロンとの間を満たしている細胞外液と脳脊髄液は平衡状態にある．特に炎症が起これば素通りと言っていいほどだ」とは高瀬先生の言葉であった．「神経細胞死に伴って細胞外腔に拡散したタウは（局所で凝集しなければ）脳脊髄液腔に達するのではないか」というシナリオを描いてみた．肝細胞障害によって細胞内酵素である GOT や GPT が血中に遊離し増加するのと同じである．1993 年も終わりに近い頃，当時の東北大学老年科佐々木英忠教授から新入医局員として出入りを許され，アルツハイマー病患者から本格的に脳脊髄液検体の収集を開始することになった．すでに 38 歳になっていた．1997 年には，東北大学 WHO 協力センター主催の「アルツハイマー病に対する新戦略」会議を，本邦からは今堀和友先生，井原康夫先生，岩坪威先生，浦上克哉先生など，海外からは，John Trojanowski 先生，Virginia Lee 先生，Rachelle Doody 先生，Christopher Clark 先生をお呼びして開催した（図 2）．この会議では米国のアルツハイマー病臨床との落差を実感させられたが，本邦での脳脊髄液バイオマーカー研究を米国に伝える形ともなり，その後，私が 1999 年に行われた第 1 回 Mild cognitive impairment 会議に招聘されるきっかけともなった．この第 1 回 Mild cognitive impairment 会議では，欧米の研究者がアルツハイマー病前駆状態についての突っ込んだ議論を展開しているのを目の当たりにし，本邦の認知症臨床レベルがいかに Behind であるかを痛感させられた．その頃の日本では，「治療医薬もない疾患に早期診断など不必要である」というバカげた意見が堂々とまかり通っていた時代であった．もう 1 つ我々に脳脊髄液バイオマーカー研究を可能ならしめた背景として，ほぼ同時期にベルギーで Innogenetics というベンチャー企業が立ち上がったことの意義は大きい．この企業には Dr. Vanmechelen や Dr. Vanderstichele などの優秀な研究者がいて，脳脊髄液中に pg/ml オーダーで存在するタウや Aβ を捕捉する優れた抗体の作成に成功していた．彼らの測定系は世界標準規格として ADNI でも採用となっている普遍性の高いものである．反面，脳脊髄液バイオマーカー研究は 2 つの欠点を有することも指摘されている．1 つは，バイオマーカーを採取する際の Invasiveness（侵襲性）の

WHO collaborating Center Meeting in Sendai, 1997

図2. 1997年仙台で開催されたWHO Collaborating Center会議「Well being in Aging」へお呼びした日米の研究者

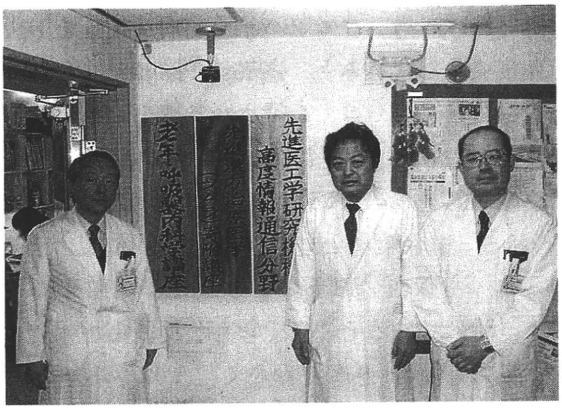

図3. 2003年10月，先進漢方治療医学講座の設置を記念して，左から，佐々木英忠教授（当時），工藤幸司教授と私

問題である．1998年に出版された米国のReagan Institute & National Institute on Aging合同コンセンサスレビューにおいても，脳脊髄液採取はmoderately invasive methodとされている．このレビューでは，理想的なバイオマーカーはnon-invasiveでなければならないとしている．2つ目は，脳脊髄液バイオマーカーは，部位情報に欠けるということである．脳脊髄液タウが上昇していても，そのタウはどの部位のNeuron　Deathを反映しているものなのかわかわない．これらの欠点を補い，なお且つ理想的なバイオマーカーに最も近いとされているのが，分子イメージング技術である．脳に蓄積したAβをPETを用いて画像化する研究は，現在東北大学未来医工学治療開発センターの工藤幸司教授がBF研究所時代の1997年から先駆的な開発を行っているものである．2000年から現在東北大学機能薬理学分野准教授の岡村信行君を派遣し，ともに開発を進めてきた（図3）．2005年までにBF-227プローブの前臨床試験を終了し，2005年から正常ボランティアそしてアルツハイマー病患者での撮像を開始した．わずか数mlのアイソトープラベルしたプローブを静注するだけで，PETカメラでアミロイド蓄積の程度とその空間的分布が把握できる優れた方法である．かつて，ガン診断にFDG-PETを提唱し実学研究を重視してきた東北大学ならではの伝統芸と言えよう．この研究の最大のハードルはプローブの開発にある．βシート構造をとった凝集Aβに選択的に結合し，且つ血液-脳関門を容易に透過し，標的がなければ脳から速やかにクリアランスされ，さらに標識体は母化合物の特性を損なわない，などの複数の優れた特性を有するプローブを見いださなければならない．これまでの経験では，いくつかの特性のうち一方の特性を上げると別の特性が下がるといったまさに微妙な匙加減を見ながらの創薬となる．しかし，いったん優秀なプローブが開発できれば，臨床サイドのハードルは脳脊髄液採取に比して遥かに低いため，市場獲得競争の激化が予想される．現在のところ，米国産の18F-AV45が近未来市場を制する勢いとなっている．

2001年，文部科学省のいわゆる「医学教育モデルコア・カリキュラム」が改定となり，「和漢薬を概説できる」という一文が加わったことにより，明治維新以来の懸案であった「医学教育の中で我が国の伝統医学である漢方医学を教える」ことが実現することとなった．2003年10月，ツムラ（株）からの寄付講座として設置が決まった「先進漢方治療医学講座」を担当することになった．時限付きの寄付講座でどんな研究ができるのだろうか．自分は師匠のもとで漢方医学を学んだこともなく，漢方専門医でもない．内心は穏やかではなかった．漢方方剤によるアルツハイマー病の新しい治療法の開発とAβ蛋白の凝集抑制作用および脱重合作用を有する漢方生薬の探索的研究にフォーカスを絞った．2005年，抑肝散というそれまでは無名に近かった漢方薬が認

知症に伴う精神症状や問題行動を改善することを見出した．幸運という以外ない．さらに，抑肝散を構成する重要な生薬である釣藤鈎は，チオフラビンT法，原子間力顕微鏡下観察，電気泳動法などによりAβ蛋白の凝集を用量依存的に抑制し，また凝集したAβ蛋白の脱重合も引き起こすこともわかった．さらに釣藤鈎をアルツハイマー病モデルマウス（Tg2576）に3ヶ月間服用させると脳内の老人斑数やAβ42濃度が減少することもわかった．このような作用は，牡丹皮という別個の生薬にもあることもわかった．今後は，精神症状や問題行動の改善効果を指標にして，抑肝散のプラセボを対象とした無作為化比較試験がぜひ必要と思われる．

現在アルツハイマー病の根本的分子標的治療の開発が米国企業を中心に飛躍的に進んでいる．今日市場化されているsymptomatic treatmentの治験は概ね6ヶ月程度で終了できるものであったが，今後開発が予定されているアルツハイマー病の進行を遅らせるためのDisease-modifying drugsの治験では，十分な薬効を確認するには少なくとも1年或いはそれ以上の治験期間が必要になると予想される．そのため，治験の長期化とコスト増が避けられない．疾患の病理像を反映し，その進行を追えるような簡便なバイオマーカーを開発しておくことは，長期に及ぶ臨床試験では不可欠である．このような考えに立って米国で2005年から発案・開始された観察式臨床試験が，Alzheimer's Disease Neuroimaging Initiative（ADNI）である．ADNIは米国，欧州，オーストラリアと本邦の世界4極で同一プロトコールを用いて実施される非ランダム化長期観察研究である．日本のADNI（J-ADNI）では，認知症医療に実績を持つ全国の38施設の協力を得て，300名のMCIを3年間，150名の早期ADを2年間，150名の正常者を3年間追跡することが計画された．プロジェクトリーダーは，東京大学の岩坪威教授である．研究の目的は，1）AD，MCI，正常高齢者において，MRIやPETなどの画像データの長期的変化に関する一定の基準値を作るための方法論を確立すること；2）画像サロゲートマーカーの妥当性を証明するために臨床指標，心理検査，血液･脳脊髄液バイオマーカーを並行して収集すること；3）Disease-modifying drugの治療効果を評価するための最良の方法を確立することの3点である．そのための観察項目として，1）MCIからADへのRate of Conversion；2）全脳，海馬などのMRI measuresのRate of volume change；3）血液・脳脊髄液バイオマーカーのRate of change；4）FDG-PETにおけるブドウ糖代謝のRate of changeを優先的の解析することになる．また，Optionalであるが，アミロイドPETにおけるアミロイド蓄積のRate of changeが加味されるであろう．ADNIは2005年末，前述のJohn Trojanowski氏によって筆者や岩坪威氏らに正式に打診がなされた．米国は治験効率やコスト削減の面からの熟慮と議論を重ねた結果「認知機能検査ベースからバイオマーカーベースへ」と大きく舵を切ったことになる．これを無視することは今後日本の医療にとって決して得策とは思えない．なぜなら，根本的治療薬の多くは米国の製薬メーカーの手によって開発が進んでいる一方で，日本は独自の新薬臨床試験を行うことが義務付けられているからである．米国で安全で有効性の高い根本的治療薬が発売になったと聞けば，日本の患者とその家族はその使用を求め医療機関に相談に訪れるであろう．その時にJ-ADNIの実施なくしては，近未来に迫る根本的治療薬の到来を（日本だけが）享受できない惨めな現実を突き付けられる恐れがある．現にNMDA受容体阻害薬であるメマンチンを使用できない国は，日本と北朝鮮のみとのことである．今こそ，経済産業省も厚生労働省も学会も医師会もともに知恵を絞り，J-ADNIをどのように支援し実りある成果が得られるかを真剣に考えるべき時であろうと思われる．

2008年，私の担当する東北大学医学系研究科所属の老年病態学分野と東北大学加齢医学研究所所属の呼吸器病態学分野が配置交換となった．このことにより，東北大学における認知症研究は加齢医学研究の一環として東北大学加齢医学研究所を中心に進められることになり，我々はその新規の臨床系研究分野として今日に至っている．本研究所では加齢医学を「生命の一生，すなわち受精から発生・成長・成熟・老化のすべての過程を扱う時間軸に沿った医

学・生物学研究」と定義している．21世紀の大きな社会的ニーズである「加齢をベースに発症する認知症など神経疾患および難治性の癌の克服」が，本研究所の今日的ミッションとされる．以上，第28回日本認知症学会学術集会を会長として仙台で開催するにあたり，「私の認知症研究」と題してやや個人的な研究史を語らせていただいた．会員の皆様のお役に立てれば幸いである．今日までの研究を支援していただき，また大所高所からの貴重なご意見をいただいた今堀和友先生，佐々木英忠先生，井原康夫先生，田平武先生，葛原茂樹先生，岩坪威先生，山口晴保先生，秋山治彦先生，樋口進先生，谷内一彦先生，浦上克哉先生，三浦雅一先生などに感謝申し上げます．また，当研究室から育っていった樋口真人君，松井敏史君，岡村信行君，田代学君，丸山将浩君らが立派に成長することを祈っております．最後に，事務局を運営いただい東北大学の古川勝敏先生，工藤幸司先生，冨田尚希先生，老年科OBの先生方に御礼申し上げます．

My contribution to dementia research

Hiroyuki Arai
Institute of Development, Aging and Cancer, Tohoku University Division of Brain Sciences Department of Geriatrics and Gerontology

In my presidential lecture at 28th Annual meeting of Japan Society for Dementia Research, I would like to address the question of "What have you done in dementia research ? I believe that I have done three major original contributions ; no more than this and no less than this.

1) Development of cerebrospinal fluid tau as a potential biomarker of Alzheimer's disease.
2) Benefits from Yokukansan, a traditional Chinese medicine, as a BPSD-reducing agent.
3) Development of BF-227 as an imaging probe of brain amyloid.

I would like to express special thanks to Prof. Trojanowski JQ and Prof. Lee VMY at The University of Pennsylvania.

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ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Effects of dehydroepiandrosterone supplementation on cognitive function and activities of daily living in older women with mild to moderate cognitive impairment

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Aim: There is little evidence that dehydroepiandrosterone (DHEA) has beneficial effects on physical and psychological functions in older women. We investigated the effect of DHEA supplementation on cognitive function and ADL in older women with cognitive impairment.

Methods: A total of 27 women aged 65–90 years (mean ± standard deviation, 83 ± 6) with mild to moderate cognitive impairment (Mini-Mental State Examination, MMSE; 10–28/30 points), receiving long-term care at a facility in Japan were enrolled. Twelve women were assigned to receive DHEA 25 mg/day p.o. for 6 months. The control group ($n = 15$) matched for age and cognitive function was followed without hormone replacement. Cognitive function was assessed by MMSE and Hasegawa Dementia Scale-Revised (HDS-R), and basic activities of daily living (ADL) by Barthel Index at baseline, 3 and 6 months. Plasma hormone levels including testosterone, DHEA, DHEA-sulfate and estradiol were also followed up.

Results: After 6 months, DHEA treatment significantly increased plasma testosterone, DHEA and DHEA-sulfate levels by 2–3-fold but not estradiol level compared to baseline. DHEA administration increased cognitive scores and maintained basic ADL score, while cognition and basic ADL deteriorated in the control group (6-month change in DHEA group vs control group; MMSE, +0.6 ± 3.2 vs −2.1 ± 2.2, $P < 0.05$; HDS-R, +2.8 ± 2.8 vs −0.3 ± 4.1, $P < 0.05$; Barthel Index, +3.7 ± 7.1 vs −2.7 ± 4.6, $P = 0.05$). Among the cognitive domains, DHEA treatment improved verbal fluency ($P < 0.05$).

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Conclusion: DHEA supplementation in older women with cognitive impairment may have beneficial effects on cognitive function and ADL. **Geriatr Gerontol Int 2010; 10: 280–287.**

Keywords: activities of daily living, cognitive function, dehydroepiandrosterone.

Introduction

Dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are the most abundant circulating steroids mainly produced by the adrenal zona reticularis in both sexes.[1] Their circulating levels decline with advancing age,[1–4] and there has been growing public interest in DHEA supplementation to prevent age-associated physical and cognitive impairment. DHEA is considered a crucial precursor of human sex steroid biosynthesis, and to exert indirect androgenic and estrogenic effects following conversion into smaller amounts of testosterone and estradiol.[5,6] While this conversion contributes to a part of testosterone production in men, its role may be much more significant in postmenopausal women whose ovarian production of androgen and estrogen has waned. Importantly, postmenopausal women with intact ovaries continue to produce androgens; DHEA(-S), testosterone and androstenedione, while their production of estradiol is minimal.[7] However, the role of androgens in older women's health is not fully understood.

Clinical trials of the effects of estrogen replacement therapy on cognitive function have shown a lack of efficacy in postmenopausal women initiating hormone replacement therapy after the age of 65 years.[8,9] On the other hand, previous reports have suggested that DHEA may have neuroprotective effects, and the age-associated DHEA(-S) decline is associated with cognitive impairment in older women.[2,10–12] One longitudinal study observed lower DHEA-S levels in patients who subsequently developed Alzheimer's disease.[13] However, controlled trials with DHEA supplementation have failed to show beneficial effects on cognition in healthy middle-aged to older women.[14–16] In these studies, the participants were limited to those who did not have cognitive impairment; therefore, it is reasonable to hypothesize that DHEA supplementation may be effective in much older women with cognitive decline as well as lower DHEA levels.

Dehydroepiandrosterone deficiency is also considered to be involved in the development of physical frailty.[17] Clinical experience with DHEA supplementation in older women is limited, and the few clinical trials examining its effect on physical function and activity of daily living (ADL) have yielded inconsistent results.[18–20] Evidence is lacking for much older women in whom physical impairment becomes more apparent and is accompanied by an age-associated DHEA decline. In our previous study, plasma DHEA and DHEA-S levels, but not estradiol level, were independently related to higher basic ADL in older women aged 70–93 years with functional decline receiving long-term care.[21] We hypothesized that in older women, DHEA replacement could be effective for the age-related decline of physical as well as psychological function.

This study therefore examined the effect of relatively low-dose (25 mg daily) p.o. DHEA supplementation for 6 months on cognitive function and ADL in older women with cognitive impairment.

Methods

Subjects and study design

In this open, non-randomized controlled study, 27 women aged 65 years or older who attended a health service facility for the elderly (a facility that provides nursing care and rehabilitation services to elderly people with disability, Mahoroba-no-Sato, located in Nagano Prefecture, Japan) were enrolled. The participants were in a chronic stable condition and receiving Long-term Care Insurance service either for admission to the facility or day-care services. The principal inclusion criteria were mild to moderate cognitive decline; both Mini-Mental State Examination (MMSE)[22] and Hasegawa Dementia Scale-Revised (HDS-R)[23] scores were between 10 and 28. The subjects were diagnosed as having a mild cognitive impairment[24] or Alzheimer's disease according to the Diagnostic and Statistical Manual of Mental Disorders IV.[25] The participants had never been treated with hormone replacement therapy, and plasma DHEA-S concentration was less than 3.0 μmol/L. The exclusion criteria were history of stroke, extremely low ADL status (Barthel Index[26] <50), malnutrition (serum albumin <3.5 mg/dL), malignancy, acute inflammation (fever, white blood cell count >10 000/μL, or other signs of infection within 4 weeks before enrollment) and overt endocrine diseases, because these diseases may affect both plasma sex hormone levels and functions. None of the subjects were taking a cholinesterase inhibitor (donepezil hydrochloride) or glucocorticoid, opiate or hormone supplement.

Twelve women were assigned to receive DHEA capsule (25 mg/day, Athena Clinics International,

Honolulu, HI, USA) and 15 women were followed up without any additive medication. Medications that could influence cognitive function and plasma hormone levels were not changed during the study period. Outcome measures were cognitive function, ADL, plasma hormone levels, blood cell counts, blood chemical parameters and subjective adverse events. They were assessed at baseline, and after 3 and 6 months. The institutional review board of Mahoroba-no-Sato approved the study protocol, and all participants or their families gave written informed consent.

Hormone measurements

Blood samples were obtained from the participants in the morning after an overnight fast, and plasma hormone levels in addition to blood cell counts and blood chemical parameters were determined by a commercial laboratory (Health Sciences Research Institute, Yokohama, Japan). DHEA and DHEA-S were assayed using sensitive radioimmunoassays with minimum detection limits of 0.04 ng/mL (0.14 nmol/L) and 2.0 μg/dL (0.05 μmol/L), respectively. Total testosterone and estradiol were assayed using chemiluminescent immunoassays with minimum detection limits of 7 ng/dL (0.2 nmol/L) and 4 pg/mL (14.7 pmol/L), respectively. The intra-assay coefficients of variation for these measurements were less than 5%.

Cognitive function

Trained examiners administered two standardized cognitive function tests, MMSE[22] and HDS-R,[23] to assess multiple, diverse aspects of cognitive function at baseline and at the 3- and 6-month visits. Both scores range 0–30, with higher scores indicating better performance. HDS-R includes questions about the subject's age, orientation, immediate recall, serial subtraction of 7 s, reciting digits backward, recalling three words, recalling five objects and word fluency (generating names of vegetables). MMSE evaluates five aspects of cognition: (i) orientation; (ii) registration; (iii) attention and calculation; (iv) recall; and (v) comprehension of spoken language (naming objects, spoken language ability, following commands). MMSE, but not HDS-R, includes four performance tests: (i) three-stage command; (ii) reading and following a command; (iii) writing; and (iv) construction drawing). Based on the results of HDS-R and MMSE, we evaluated seven cognitive domains (points) as follows: (i) orientation (10); (ii) verbal memory (9); (iii) attention and calculation (5); (iv) visual memory (5); (v) spoken-language comprehension (9); (vi) verbal fluency (5); and (vii) performance (7).

Other functional parameters and anthropometric measures

Trained nurses and physical therapists visited the participants at the facility and performed the assessments. Basic ADL was assessed by Barthel Index,[26] mood by Geriatric Depression Scale (GDS, 15 items),[27] and ADL-related vitality by Vitality Index (10-point scale).[28] Higher GDS scores indicate a more marked self-reported depressive status, while higher Vitality Index scores indicate greater willingness.

Adverse events

Information regarding adverse events was obtained by questioning or examining the subjects. At each visit during the treatment period, all new complaints and symptoms were recorded. The safety of DHEA supplementation was assessed from the symptoms and by measuring blood chemical parameters including liver and kidney function, electrolyte levels and hematological parameters. Preexisting complaints or symptoms that increased in intensity or frequency during the treatment period also were examined.

Statistical analysis

Data were analyzed using SPSS statistical software ver. 17.0. Changes in outcome measures at 3 and 6 months were calculated by comparing the values at baseline with those at each measurement. Within each group, the significance of the change from baseline to 6 months was tested using paired Student's *t*-test. Repeated-measures ANOVA was used to test the statistical significance of the effects of DHEA versus control. Significance tests were two-sided, with an α-level of 0.05.

Results

Hormone changes and adverse effects

Characteristics and hormone levels at baseline according to treatment groups are shown in Table 1. There were no significant differences between the DHEA group and the control group in age, length of education, nutritional parameters, functional parameters and plasma hormone levels. DHEA supplementation was well tolerated, with high adherence, and there were no detectable adverse events and none of the subjects dropped out during the study. Measures of liver function, kidney function, electrolyte levels and hemoglobin level were not significantly altered by treatment with DHEA (data not shown). Body mass index remained unchanged in both groups.

Subjects in the DHEA group showed a significant increase from baseline to 3 and 6 months in levels of

Table 1 Participant characteristics at baseline

	DHEA	Control
No. of subjects	12	15
Age, years	82 ± 6 (69–90)	83 ± 6 (65–89)
Education, years	8 ± 2	8 ± 2
Nutritional parameters		
Body mass index, kg/m^2	22.0 ± 2.4 (18.8–26.4)	22.4 ± 3.2 (17.6–27.1)
Albumin, g/dL	4.4 ± 0.3 (3.7–4.9)	4.3 ± 3.2 (3.8–4.7)
Total cholesterol, mg/dL	227 ± 39 (166–294)	203 ± 22 (173–250)
Functional parameters		
MMSE	24.0 ± 4.2 (18–28)	23.4 ± 4.4 (14–28)
HDS-R	19.9 ± 5.8 (10–28)	21.7 ± 5.6 (10–28)
Barthel Index	89.6 ± 9.4 (55–100)	89.7 ± 6.4 (75–100)
Vitality Index	9.8 ± 0.6 (8–10)	9.9 ± 0.3 (9–10)
GDS	7.0 ± 4.4 (1–15)	7.0 ± 4.0 (1–13)
Hormones		
DHEA-S, μmol/L	1.8 ± 0.6 (0.7–2.4)	1.6 ± 0.8 (0.3–2.9)
DHEA, nmol/L	7.6 ± 4.7 (2.4–19.1)	6.6 ± 3.1 (2.1–11.5)
Testosterone, nmol/L	1.4 ± 0.4 (0.9–2.3)	1.3 ± 0.9 (0.2–3.8)
Estradiol, pmol/L	88 ± 52 (15–187)	70 ± 26 (45–115)

Values are shown as mean ± standard deviation (range). HDS-R, Hasegawa Dementia Scale-Revised; MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale; DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone. There was no significant difference in each parameter between the groups.

circulating DHEA, DHEA-S and testosterone, with levels reaching approximately 2–3-fold higher than those at baseline, whereas the increase in estradiol level was not significant (Table 2). Subjects in the control group showed no significant change in hormone levels.

Changes in cognitive function and ADL

The changes in functional parameters in each group from baseline to 6 months are shown in Table 2. After 6 months, mean HDS-R score significantly improved in the DHEA group while it remained unchanged in the control group. Mean MMSE score significantly declined in the control group while it remained unchanged in the DHEA group. As a result, significant differences were found in these scores between the groups. DHEA treatment maintained Barthel Index score, whereas the score deteriorated significantly during 6 months in the control group, although the between-group difference at 6 months was not statistically significant. Regarding the components of Barthel Index, in the control group, the sum score of mobility deteriorated significantly after 6 months compared to baseline, while no significant change was observed in the sum score of self care (Table 3). Neither Vitality Index nor GDS changed significantly in both groups.

Table 4 shows the cognitive domain scores at baseline and at 3- and 6-month follow up. Among the seven cognitive domains, DHEA treatment improved verbal fluency ($P < 0.05$), resulting in a significant difference at 6 months between the groups. Verbal memory showed a non-significant trend towards improvement in the DHEA group. Performance test scores significantly declined over time in both groups. There were no differences between the groups in the scores of orientation, attention and calculation, visual memory and spoken-language comprehension.

Discussion

Daily administration of DHEA 25 mg for 6 months in elderly women with mild to moderate cognitive impairment improved cognitive function and maintained basic ADL, compared to the control group. Among the cognitive domains, DHEA significantly improved verbal fluency. At baseline, DHEA and DHEA-S levels were lower than those reported in healthy postmenopausal women in both groups,[2,4] and DHEA treatment increased DHEA, DHEA-S and testosterone levels by 2–3-fold to the mid-normal range for premenopausal

Table 2 Changes in hormone levels and functional parameters by treatment group

	DHEA				Control				*P*
	Baseline	3 months	6 months	0–6-month difference	Baseline	3 months	6 months	0–6-month difference	
Hormones									
DHEA-S, μmol/L	1.8 ± 0.6	4.5 ± 1.3*	5.6 ± 2.9*	3.8 ± 2.8	1.6 ± 0.8	1.8 ± 1.0	1.7 ± 0.8	−0.02 ± 0.4	<0.01
DHEA, nmol/L	7.6 ± 4.7	12.2 ± 4.8*	13.7 ± 7.7*	6.1 ± 8.2	6.6 ± 3.1	7.3 ± 3.7	7.4 ± 4.5	0.9 ± 2.8	0.04
Testosterone, nmol/L	1.4 ± 0.4	2.3 ± 0.7*	2.3 ± 0.8*	0.9 ± 0.8	1.4 ± 0.7	1.4 ± 0.7	1.6 ± 0.8	0.2 ± 0.5	<0.01
Estradiol, pmol/L	88 ± 52	92 ± 48	101 ± 37	13 ± 51	70 ± 26	68 ± 20	67 ± 42	−4.0 ± 38	0.17
Functional parameters									
MMSE	24.0 ± 4.2	24.1 ± 4.6	24.6 ± 4.3	0.6 ± 3.2	23.4 ± 4.4	23.1 ± 5.4	21.3 ± 5.0**	−2.1 ± 2.2	0.04
HDS-R	19.9 ± 5.8	20.5 ± 7.3	22.7 ± 6.3**	2.8 ± 2.8	21.7 ± 5.6	22.1 ± 5.6	21.3 ± 6.4	−0.3 ± 4.1	0.04
Barthel Index	89.6 ± 9.4	92.7 ± 6.5	93.3 ± 6.8	3.7 ± 7.1	89.7 ± 6.4	86.9 ± 7.2	87.0 ± 6.7*	−2.7 ± 4.6	0.04
Vitality Index	9.8 ± 0.6	9.7 ± 0.5	9.7 ± 0.7	−0.1 ± 1.0	9.9 ± 0.3	9.8 ± 0.5	9.7 ± 1.0	−0.3 ± 1.0	0.80
GDS	7.0 ± 4.4	6.2 ± 3.4	6.6 ± 3.7	−0.4 ± 1.7	7.0 ± 4.0	8.3 ± 3.9	7.5 ± 3.5	0.5 ± 3.3	0.60

Values are shown as mean ± standard deviation (range). *P*-values are for repeated-measure ANOVA over all three time points. DHEA, dehydroepiandrosterone; HDS-R, Hasegawa Dementia Scale-Revised; MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale; DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone. **$P < 0.01$ compared to baseline, *$P < 0.05$ compared to baseline.

women.[2] No detectable adverse effects were observed throughout the study.

According to the previous trials, DHEA supplementation of 50 mg or more daily does not provide beneficial effects on cognition in healthy middle-aged to elderly women without cognitive impairment.[14–16] However, in a small-scale randomized double-blind placebo-controlled study, DHEA transiently improved cognition (after 3 months) in subjects with Alzheimer's disease while the improvement was not significant at 6 months.[29] Preliminary analysis of the small number of subjects in the present study suggested that DHEA treatment was no less effective in subjects with low baseline cognitive function than those with higher cognitive function (data not shown). Whether the effects of DHEA might be influenced by baseline cognitive function should be further investigated.

It is noteworthy that the 6-month effect of donepezil hydrochloride (5 or 10 mg), the only cholinesterase inhibitor used in Japan, in patients with Alzheimer's disease ranged from no change to less than 1 point improvement in MMSE score,[29–33] which is not so different from the effect of DHEA observed in the present study.

In the present study, not only the participants' cognitive function was impaired, but baseline plasma DHEA(-S) level was also low compared to that in postmenopausal or perimenopausal women.[2,4,10] Regarding DHEA-S levels, according to a report in which healthy pre- and postmenopausal women were studied, DHEA-S levels in women aged 35–44 years and 45–55 years were as follows: 4.31 ± 2.11, 3.90 (mean ± standard deviation) and 3.42 ± 2.01 μmol/L.[2] In this study, DHEA-S was measured using chemiluminescent enzyme immunometric assay; although the measurements by this method and those by radioimmunoassay have been reported to be comparable. In our study, DHEA treatment increased DHEA-S levels to the mid-normal range for premenopausal women.[2] Also, the subjects with lower baseline DHEA-S levels showed non-significant trend towards more improvement in cognitive scores (data not shown). Thus, future studies are needed to explore whether the effects of DHEA might be influenced by baseline DHEA levels.

Because the DHEA receptor has not been identified, DHEA may act after conversion to testosterone and subsequently estradiol through estrogen receptors and androgen receptors, both of which are found in the hippocampus and frontal lobes and subserve verbal memory and working memory in women.[34,35] Further, hippocampal volume and perfusion have been shown to correlate with serum DHEA-S level in demented patients.[36,37] It has also been suggested that estrogenic and androgenic derivatives of DHEA might have different effects on cognitive functions.[38] However, the mechanism by which DHEA improves cognitive

Table 3 Changes in mobility and self-care scores in Barthel Index during the study

Domains (points)	Mean ± SD Baseline	3 months	6 months	Change (0–6 months)	*P*
Mobility (55)					
DHEA	46.9 ± 9.2	48.2 ± 6.0	49.2 ± 5.2	2.3 ± 5.4	0.01
Control	47.5 ± 5.4	46.2 ± 5.5	45.0 ± 4.3*	−3.7 ± 3.9	
Self care (45)					
DHEA	42.7 ± 6.1	44.5 ± 1.5	43.1 ± 2.5	0.4 ± 6.9	0.96
Control	41.8 ± 4.2	42.5 ± 3.4	41.2 ± 4.3	0.7 ± 3.2	

Mobility is the sum score of five domains: (i) transfer (moving from a bed to a wheelchair and back); (ii) walking on a level surface; (iii) propelling a wheel chair; (iv) ascending and descending stairs; and (v) bathing and toilet use. Self care includes feeding, grooming, dressing, bowels and bladder. *P*-values are for repeated-measure ANOVA over all three time points. $^{*}P < 0.05$ compared to baseline. SD, standard deviation.

Table 4 Changes in cognitive domain scores during study

Domains (points)	Mean ± SD Baseline	3 months	6 months	Change (0–6 months)	*P*
Orientation (10)					
DHEA	8.3 ± 1.9	8.0 ± 2.7	7.5 ± 3.0	−0.1 ± 1.2	0.28
Control	8.3 ± 1.9	8.0 ± 2.8	7.5 ± 2.9	−0.7 ± 1.7	
Verbal memory (9)					
DHEA	5.7 ± 2.1	6.5 ± 2.3	6.7 ± 2.5†	1.0 ± 1.9	0.79
Control	6.5 ± 1.7	7.5 ± 1.8	7.0 ± 1.9	0.5 ± 1.7	
Attention and calculation (5)					
DHEA	2.3 ± 1.9	2.8 ± 2.0	2.7 ± 1.8	0 ± 2.3	0.79
Control	2.0 ± 1.7	1.9 ± 1.2	1.8 ± 1.5	−0.5 ± 1.4	
Visual memory (5)					
DHEA	3.6 ± 0.9	3.6 ± 1.3	3.8 ± 1.2	0.3 ± 1.1	0.91
Control	3.6 ± 1.3	3.9 ± 0.9	3.9 ± 1.0	0.5 ± 1.1	
Language comprehension (9)					
DHEA	8.5 ± 0.8	7.8 ± 2.5	8.7 ± 0.7	0.1 ± 0.3	0.12
Control	8.5 ± 0.8	8.5 ± 0.8	8.4 ± 1.1	−0.1 ± 0.9	
Verbal fluency (5)					
DHEA	2.8 ± 3.3	2.5 ± 2.0	4.3 ± 1.1*	1.5 ± 1.7	0.01
Control	3.2 ± 1.9	3.8 ± 1.6	3.3 ± 1.9	0.1 ± 2.1	
Performance (7)					
DHEA	5.7 ± 0.7	5.5 ± 0.7	4.8 ± 0.4**	−0.8 ± 0.6	0.36
Control	5.6 ± 0.6	5.1 ± 0.6	4.5 ± 0.9**	−1.1 ± 0.8	

Change refers to score change during 0–6 months for each parameter in each treatment group. *P*-values are for repeated-measure ANOVA over all three time points. DHEA, dehydroepiandrosterone. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.1$ vs baseline. SD, standard deviation.

function is unknown. In the present study, plasma estradiol level was not significantly increased after DHEA treatment, implying that its beneficial effects on cognition might be androgen-dependent. Unfortunately, free testosterone levels were not measured, because they were considered to be undetectable in many cases in older women. In addition, sex hormone-binding globulin (SHBG) measurement was not available; however, it has been reported that DHEA 50 mg treatment for 3 months in postmenopausal women did not significantly change SHBG levels,[39] suggesting that the change in SHBG-bound hormone levels after DHEA treatment might be minimal. Given the local aromatization of androgen to estradiol in the brain, the effect of DHEA on cognition might be indirect, complex and heterogeneous. The molecular mechanism underlying the association

between DHEA and cognitive function needs to be clarified, and active forms of testosterone and estradiol should also be examined to investigate whether they would change after DHEA administration.

In our previous study, plasma DHEA and DHEA-S levels were independently related to higher basic ADL in older women aged 70–93 years with functional decline,[21] and other reports have shown a correlation between DHEA level and muscle mass, strength and physical performance.[40,41] In the present study, DHEA treatment maintained the Barthel Index score, while the score deteriorated significantly in the control group. Regarding body composition and strength, DHEA administration in postmenopausal older women aged up to 80 years did not alter body composition, physical performance or strength.[18–20] However, in one small-scale open-label trial, DHEA treatment for 4 weeks improved ADL in three out of seven patients (both men and women) with multi-infarct dementia.[42] All these studies are preliminary, and large-scale and long-term studies are required to ascertain whether DHEA could have a beneficial effect on ADL in older women.

In the present study, no effect of DHEA on depressive mood or vitality was observed, consistent with most clinical trials in older women.[15,43,44] This might be attributable to the participants' relatively low depressive status and high vitality status, namely, ceiling effects.

The limitations of our study should be acknowledged. First, this study was neither blinded nor randomized. Second, the number of participants was too small to confirm the results. Thus, results need to be confirmed by large-scale randomized trials to exclude possible selection bias. Third, considering the sensitivity and accuracy, a standard test like the Alzheimer's Disease Assessment Scale should be used in clinical trials to ascertain the effect of DHEA. Finally, our study duration was 6 months so it does not provide any information on the effects of longer-term DHEA supplementation.

In summary, this small study showed that supplementation of DHEA 25 mg for 6 months to older women with mild to moderate cognitive impairment improved cognitive scores and maintained basic ADL. The results should be confirmed in large-scale randomized trials.

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