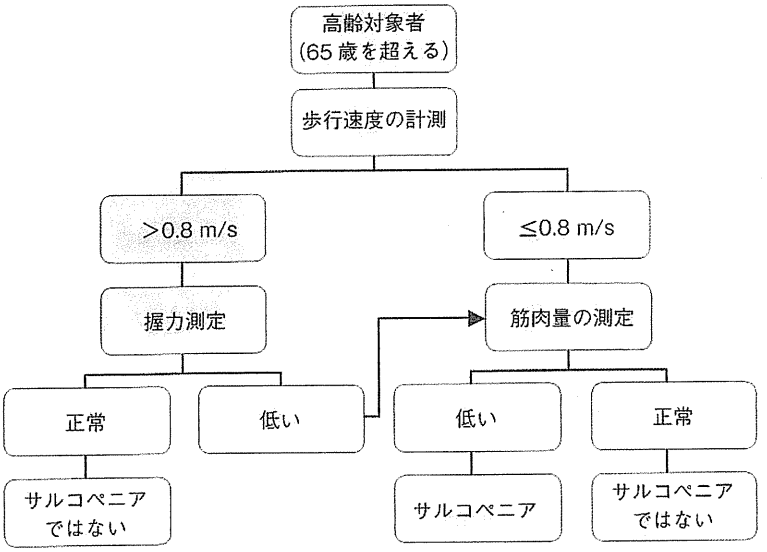

図5　高齢者におけるサルコペニアの発見のためのアルゴリズム

わたる(図4)．しかも，これらは複合して関わるため，1つひとつを区別して要因を分析することが難しい．

加齢性筋肉減少症(サルコペニア)

サルコペニアとは高齢者が虚弱(心身の機能低下)過程で全身，特に四肢の筋肉が量的，質的に低下することを指し，その結果，歩行機能をはじめとする身体機能が低下する．サルコペニアの原因や対策は世界的に注目されており，2010年にBritish Geriatrics Societyからサルコペニアの定義に関するコンセンサスレポート[1]が発表された．この中で，筋肉量の低下のみの場合“前サルコペニア(サルコペニアの前段階)”，筋肉量の低下に加えて筋力の低下もしくは身体機能の低下が認められる場合“サルコペニア”，筋肉量の低下，筋力の低下，身体機能の低下が3つとも認められる場合“重度のサルコペニア”と定義している．さらに，同コンセンサスレポートでは筋肉量をDXA法もしくは生体インピーダンス法で，筋力を握力で，身体機能を歩行速度，バランス，Up & Goテストの組み合わせで評価し，これを組み合わせて図5のような流れで判断するよう勧めている．また，サルコペニアの結果生じる事象として，日常生活自立度(基本的ADL，手段的ADL)，生活の質(QOL)，代謝・生化学・炎症マーカー，転倒，介護施設や病院への入所・入院，社会的支援，死亡率などに注目するよう勧めている．

転倒の評価方法

上記のコンセンサスレポートを加味して，われわれの施設では表1に示すような検査を行い，高齢者の易転倒性を評価している．もちろん，これらの検査は転倒リスクの評価に有用であるが，機器や人手，時間を要するなどマススクリーニングに向かない面もある．そこで，転倒のハイリスク者をより簡易な方法でスクリーニングするために考案したのが転倒スコアである．転倒スコアは自己記入式の調査票であり，身体機能に関する8項目，疾患もしくは老年症候群に関する8項目，環境要因に関する5項目の計21項目と，過去1年間での転倒歴を問う全22項目から成っている(表2)．“はい”，“いいえ”で答える二者択一形式になっており，転倒しやすい方の答えが多いほど転倒リスクが高い．地域在住高齢者を対象とした横断調査の結果，「つまずくことがある」，「信号が青の間に横断

表1　転倒評価のための検査

問診(転倒歴，ADL，環境要因，基礎疾患，服用薬剤)
診察(身長，体重，体脂肪率，血圧，下腿最大周囲径)
視力
下肢筋力
体組成測定
握力
片足立ち時間(開眼，閉眼)
継ぎ足歩行
手伸ばし試験
Up & Go テスト
重心動揺検査
脊椎X線
骨量測定
起立性血圧
頭部MRI
聴力・内耳機能

表2　転倒スコア

過去1年に転んだことがありますか？	(はい　いいえ)	
「はい」の場合，転倒回数(　回/年)		
1．つまずくことがありますか	(はい　いいえ)	身体機能
2．手すりを使わないと階段昇降ができませんか	(はい　いいえ)	
3．歩く速度が遅くなってきましたか	(はい　いいえ)	
4．横断歩道を青のうちに渡りきれますか	(はい　いいえ)	
5．1kmくらい続けて歩けますか	(はい　いいえ)	
6．片足で5秒くらい立つことができますか	(はい　いいえ)	
7．杖を使ってますか	(はい　いいえ)	
8．タオルは固く絞れますか	(はい　いいえ)	
9．めまい・ふらつきがありますか	(はい　いいえ)	疾患 老年症候群
10．背中が丸くなってきましたか	(はい　いいえ)	
11．膝が痛みますか	(はい　いいえ)	
12．目が見えにくいですか	(はい　いいえ)	
13．耳が聞こえにくいですか	(はい　いいえ)	
14．もの忘れが気になりますか	(はい　いいえ)	
15．転ばないかと不安になりますか	(はい　いいえ)	
16．毎日，お薬を5種類以上飲んでいますか	(はい　いいえ)	
17．家の中が暗く感じますか	(はい　いいえ)	環境要因
18．家の中によけて通るものがありますか	(はい　いいえ)	
19．家の中に段差がありますか	(はい　いいえ)	
20．階段を使わなくてはなりませんか	(はい　いいえ)	
21．生活上，急な坂道を歩きますか	(はい　いいえ)	

歩道を渡れない」，「杖の使用」，「タオルを固く絞れない」，「めまい・ふらつきがある」，「膝が痛む」，「屋内の障害物」の7項目が，調査以前の転倒歴と関連すること[2]，「過去(調査以前)の転倒歴」，「歩行速度が遅くなった」，「杖の使用」，「背中が丸くなった」，「5種類以上の服薬」の5項目が，調査後の転倒発生と関連すること[3]が報告されている．転倒スコアは，簡便かつ包括的な転倒評価方法ということができる．

骨粗鬆症と虚弱

“虚弱”は高齢者が抱える普遍的な問題であり，要介護状態を生む大きな原因である．虚弱

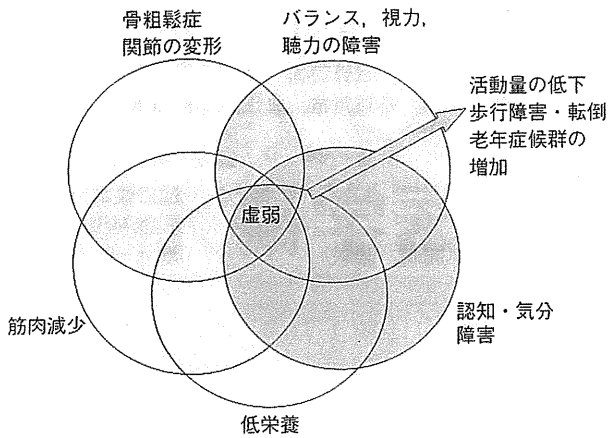

図6

とは加齢に伴って生ずる心身の脆弱な状態であり，複数の臓器・器官の機能低下に起因する．骨粗鬆症やサルコペニアはその主な要因であり，ほかに摂食・嚥下障害と関連する低栄養状態や認知・気分障害(意欲低下，うつなど)など様々な要因が関わる(図6)．虚弱は，活動量の低下，歩行障害・転倒，痩せ，そのほか老年症候群の集積をもたらす．虚弱はその多因子性ゆえ，原因を求め介入することが容易ではないが，骨粗鬆症はその中で数少ない介入可能な要因である．後述されるSeminarを基に，エビデンスに基づく評価・介入を行うことが大切である．

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ORIGINAL ARTICLE

The Earliest Stage of Cognitive Impairment in Transition From Normal Aging to Alzheimer Disease Is Marked by Prominent RNA Oxidation in Vulnerable Neurons

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Abstract

Although neuronal RNA oxidation is a prominent and established feature in age-associated neurodegenerative disorders such as Alzheimer disease (AD), oxidative damage to neuronal RNA in aging and in the transitional stages from normal elderly to the onset of AD has not been fully examined. In this study, we used an in situ approach to identify an oxidized RNA nucleoside 8-hydroxyguanosine (8OHG) in the cerebral cortex of 65 individuals without dementia ranging in age from 0.3 to 86 years. We also examined brain samples from 20 elderly who were evaluated for their premortem clinical dementia rating score and postmortem brain pathologic diagnoses to investigate preclinical AD and mild cognitive impairment. Relative density measurements of 8OHG-immunoreactivity revealed a statistically significant increase in neuronal RNA oxidation during aging in the hippocampus and the temporal neocortex. In subjects with mild cognitive impairment but not preclinical AD, neurons of the temporal cortex showed a higher burden of oxidized RNA compared to age-matched controls. These results indicate that, although neuronal RNA oxidation fundamentally occurs as an age-associated phenomenon, more prominent RNA damage than in normal aging correlates with the onset of cognitive impairment in the prodromal stage of AD.

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Key Words: 8-Hydroxyguanosine, Aging, Mild cognitive impairment, Neurodegeneration, Oxidative damage, Preclinical Alzheimer disease, RNA.

INTRODUCTION

Cells in the brain encounter a cumulative burden of oxidative and metabolic stress that may be a universal feature of the aging process as well as a major causal factor of age-related dysfunction. Macromolecules, including nucleic acids, proteins, and lipids, are oxidatively modified during aging. Indeed, the brain is particularly vulnerable to free radical damage because of its high oxygen consumption rate, abundant lipid content, and relative paucity of antioxidant enzymes compared with other organs (1, 2). Although oxidative damage to DNA is more frequently investigated than oxidative damage to RNA, cellular RNA molecules may be more susceptible to oxidative insult than DNA because of their single-stranded structure without protective histones. Moreover, ribosomal RNA molecules are located closely to mitochondria, a major source of free radicals (3). We have focused on RNA oxidation and, for the first time, reported RNA oxidation in vulnerable neurons in Alzheimer disease (AD) (4). This was followed by further in situ and biochemical studies on AD (5–10), as well as studies elucidating neuronal RNA oxidation in other age-associated degenerative disorders such as Parkinson disease (11), dementia with Lewy bodies (12), and amyotrophic lateral sclerosis (13). Although nucleic acids oxidation, predominantly in RNA, was reported in the aged rat brain (14, 15), age-associated changes in the RNA oxidation in human brain have not been investigated.

To understand the process of age-associated neurodegenerative diseases, it is important to investigate not only the underlying mechanisms of brain aging but also the transition from normality to the onset of symptomatic disease. For AD, subjects at the preclinical stage and subsequent prodromal stage of mild cognitive impairment (MCI) have become a research focus. Several previous studies have demonstrated oxidative damage to proteins, lipids, and nucleic acids in brains of MCI cases (16–21); some studies have focused on oxidative RNA damage (22, 23). A few recent studies have investigated oxidative protein modification and lipid peroxidation in brains of preclinical AD (24–27), but oxidative RNA

damage in preclinical AD has not been studied. Whether there are significant changes in levels of oxidative damage in brains of preclinical AD cases is controversial. Using a proteomic approach, Terni et al (24) showed that the mitochondrial ATP-synthase was oxidized in the entorhinal cortex at the early stages of AD-related tau pathology (Stages I/II of Braak), which correspond to a clinically silent period. Bradley et al (25) reported increased levels of 4-hydroxynonenal and acrolein in the hippocampus and decreased levels of these lipid peroxidation products in the cerebellum in preclinical AD. However, that report showed no significant changes in levels of these lipid peroxidation products in the temporal cortex and no significant changes in levels of protein carbonyls in the hippocampus, the temporal cortex, or the cerebellum. Aluise et al (26, 27) also observed no significant changes in levels of 4-hydroxynonenal, protein carbonyls, and 3-nitrotyrosine in the parietal cortex of preclinical AD cases.

In this study, we used an in situ approach to identify an oxidized RNA nucleoside, 8-hydroxyguanosine (8OHG) in the cerebral cortex of subjects from an aging series of individuals without dementia between the first and the ninth decade. We also investigated neuronal RNA oxidation in the cerebral cortex among elderly subjects at transitional stages between normality and dementia, that is, preclinical AD and MCI.

MATERIALS AND METHODS

Tissue

For Experiment 1 (neuronal RNA oxidation in aging brains), brain tissue samples were obtained at autopsy from 2 consecutive series of subjects without dementia or other neurologic disorders collected at the Department of Pathology, Case Western Reserve University. Group 1 consisted of 27 subjects (aged 3–86 years). Group 2 consisted of 38 subjects (aged 0.3–82 years). Postmortem intervals (PMIs) before fixation were 4 to 27 hours (mean ± SD = 15.6 ± 6.7 hours) in Group 1 and 3 to 30 hours (11.1 ± 7.4 hours) in Group 2. Slices of the hippocampus and the subiculum from Group 1 subjects were fixed in methacarn (methanol/chloroform/acetic acid, 6:3:1); slices of the temporal cortex including the inferior temporal gyrus or the occipitotemporal gyrus from the Group 2 subjects were fixed in phosphate-buffered formalin. After fixation, all the tissue slices were dehydrated through graded ethanols followed by xylene, and embedded in paraffin. Six-micrometer-thick sections were cut and mounted on Silane (Sigma, St Louis, MO)-coated glass slides.

For Experiment 2 (neuronal RNA oxidation in preclinical AD and MCI), a series of paraffin sections of the temporal cortex and cerebellum were obtained from the Neuropathology Core Laboratory of the Washington University Alzheimer's Disease Research Center (St Louis, MO). Cases representing 4 distinct diagnostic categories using the Clinical Dementia Rating (CDR) (28–30) were studied. Control cases were individuals with normal cognitive functions who are generally free of amyloid plaques and neurofibrillary tangles (NFTs) in the brain. Individuals with normal cognitive functions who met current pathologic criteria for AD (31) were classified as having preclinical AD. The borderline condition between normal and dementia, defined as CDR = 0.5, were classified as MCI. The level of CDR = 0.5 covers not only subjects with the core MCI but also those with "pre-MCI" and "very mild dementia" (32, 33). Subjects with MCI (CDR = 0.5) and mild dementia (CDR = 1) met pathologic criteria for AD (30). There were 5 control subjects (aged 80–95 years; PMI = 6–13 hours), 4 subjects with preclinical AD (aged 83–93 years; PMI = 2–17 hours), 6 subjects with MCI (aged 90–102 years; PMI = 6–23 hours), and 5 subjects with mild AD (aged 74–96 years; PMI = 4–13 hours) (Table). At autopsy, samples of the temporal cortex and cerebellum were fixed in phosphate-buffered formalin and embedded in paraffin. Six-micrometer-thick sections were cut and mounted on Silane-coated slides.

Immunocytochemistry and Antibodies

Immunostaining was performed in batches of 10 slides at the same time. Samples from subjects of different age decades in Experiment 1 and from subjects in all 4 diagnostic categories in Experiment 2 were included in each batch. All steps of each immunostaining were carefully controlled so that all the slides were processed with the identical solutions and incubated for identical times. After deparaffinization with xylene (3 times, 10 minutes each), sections were hydrated through a graded ethanol series (100%, 95%, 70%, and 50% ethanol, 10 minutes each). Endogenous peroxidase activity was eliminated by 30-minute incubation with 3% H_2O_2 in methanol, and nonspecific binding sites were blocked by 30-minute incubation with 10% normal goat serum in Tris-buffered saline (150 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.6). A mouse monoclonal antibody against 8OHG (1F7, 1:30; Trevigen, Gaithersburg, MD) was used to detect oxidized nucleosides (34) to detect oxidized nucleosides. After treatment with 10 μg/ml proteinase K (Boehringer Mannheim,

TABLE. Classification of 4 Categories of Subjects According to Clinical Dementia Rating Score and CERAD Pathologic Criteria

Category	CDR	Brain Pathology (CERAD)	n	Age, Mean (SD), y	PMI, Mean (SD), h
Controls	0	Normal	5	88.4 (5.7)	10.8 (3.8)
Preclinical AD	0	Definite AD	4	88.8 (4.3)	10.2 (6.2)
MCI	0.5	Definite AD (n = 5) Possible AD (n = 1)	6	93.5 (4.5)	13.7 (7.0)
Mild AD	1	Definite AD	5	85.4 (8.1)	9.2 (3.8)

AD, Alzheimer disease; CDR, Clinical Dementia Rating (28); CERAD, Consortium to Establish a Registry for Alzheimer's Disease (31); MCI, mild cognitive impairment; PMI, postmortem interval.

Indianapolis, IN) in phosphate-buffered saline (pH 7.4) for 40 minutes at 37°C, sections were incubated with the 1F7 for 18 hours at 4°C. Sections were then incubated with goat affinity-purified antibody to mouse immunoglobulin (1:50; ICN Pharmaceuticals, Aurora, OH) as secondary antibody for 30 minutes at room temperature (RT). Immunostaining was developed by the peroxidase-antiperoxidase procedure (Mouse ClonoPAP, 1:250; Covance, Gaithersburg, MD) for 60 minutes at RT (35) using 0.75 mg/ml 3,3′-diaminobenzidine substrate (Sigma) in 0.015% H_2O_2, 50 mmol/L Tris-HCl, pH 7.6 for 10 minutes at RT. Subsequently, sections were dehydrated through a graded ethanol series (50%, 70%, 95%, and 100% ethanol, 10 minutes each), cleared in xylene (3 times, 10 minutes each), and coverslipped with synthetic mounting medium (Permount; Fisher Scientific, Fair Lawn, NJ). The specificity of 1F7 was confirmed by primary antibody omission or by absorption with purified 8OHG (Cayman Chemical, Ann Arbor, MI) (4). Although 1F7 recognizes RNA-derived 8OHG as well as DNA-derived 8-hydroxydeoxyguanosine with similar binding affinities (34), we confirmed that 1F7 immunolabeling in neurons in AD is predominantly in RNA by the pretreatment with DNase or RNase (4), as well as by immunoelectron microscopy, which showed that most 8OHG is present in the endoplasmic reticulum (5). In this study, additional sections were pretreated with RNase-free DNase I (10 U/μl for 2 hours at 37°C; Roche, Mannheim, Germany) or DNase-free RNase (0.5 μg/μl for 2 hours at 37°C; Boehringer Mannheim) after proteinase-K treatment.

Relative Scale of 8OHG Immunostaining

All measurements were performed using a Q500IW-EX Image Processing and Analysis System (Leica Microsystems, Wetzlar, Germany) linked to a SONY CCD Camera (XC-75CE; Sony, Tokyo, Japan) mounted on a Nikon MICROPHOT-FX microscope (Nikon, Tokyo, Japan). Measurements were performed in the pyramidal layer of the hippocampal subiculum, the Layer III of the temporal neocortex and the Purkinje cell layer of the cerebellum. Immunoreactivity intensities were evaluated by measuring the average optical density in an area comprising the cytoplasm and nucleus, as previously described (4–7, 12, 36). To prevent possible bias during selection of the fields for analysis, fields were selected according to the following sampling criteria: for the hippocampal subiculum, we selected the pyramidal layer adjacent to the CA1 field, that is, the prosubiculum. Because an appropriate anatomic landmark was not always identified in the temporal neocortex and cerebellum, a rule for slide scanning was introduced to select the portion of Layer III of the temporal neocortex or the Purkinje cell layer of the cerebellum. The sampling was performed by moving the microscopic field from the upper left field to right along the entire length of the section, then by moving the field vertically down to successive subjacent fields and by moving the field horizontally back to the left. When we encountered each of the target neuronal cell layers, we aligned the microscopic field so that the horizontal line of the field was parallel to each of the neuronal cell layers. Then, 3 adjacent fields (each field = 460 μm × 428 μm) were acquired for image analysis. In each field of the videocamera, 5 pyramidal neurons sectioned near their equator (based on a section plane that included the nucleolus) were selected and manually outlined. The selection of the neurons within the field was performed under the same rule as the field selection within the section (upper left first). The nucleus was included for the analysis of optical density because damage to RNA was nuclear as well as cytoplasmic. Because the oxidized RNA was localized predominantly in the cytoplasm rather than nucleus in neurons (4), the area ratio of the nucleus to the cytoplasm might be a factor that influenced the neuronal optical density of 8OHG. Therefore, we selected neurons including the nucleolus so that the nucleus/cytoplasm ratio was expected to be similar among neurons of the same neuron population. The optical density measurement was obtained for each of the 3 fields and averaged. Finally, the optical density value was corrected for background by subtracting the optical density of the white matter on the same section. All measurements were done under the same optical and light conditions as well as using an electronic shading correction to compensate for any unevenness that might be present in the illumination.

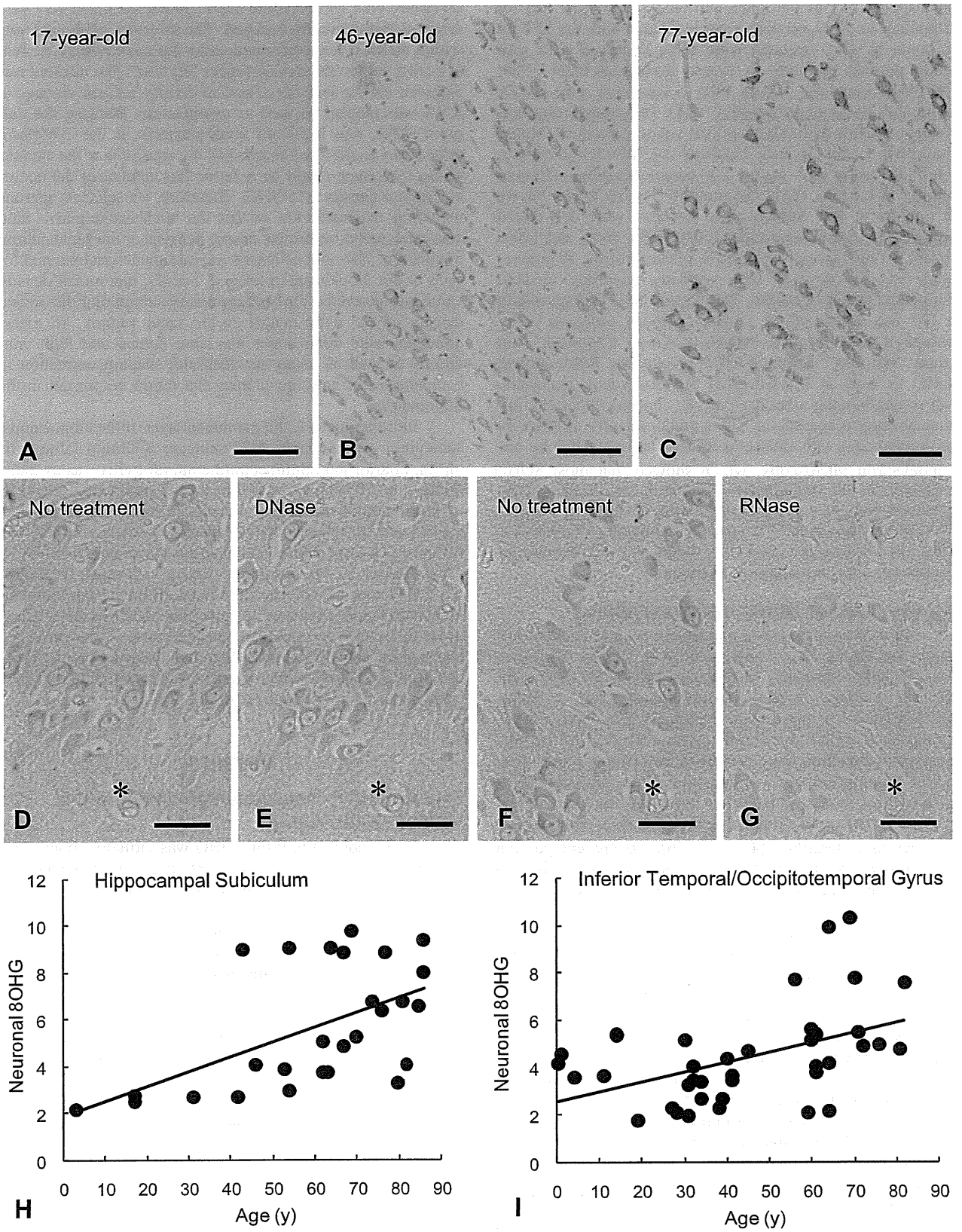
In Experiment 1, the pyramidal layer of the hippocampal subiculum was available in 27 subjects of Group 1; Layer III of the inferior temporal/occipitotemporal gyrus was available in the other 38 subjects of Group 2. In Experiment 2, Layer III of the temporal neocortex and the Purkinje cell layer were analyzed in 20 subjects. Statistical analysis was performed with linear regression analysis and analysis of variance (ANOVA), using StatView 5.0 program (Abacus Concepts, Berkeley, CA). Fisher protected least significant difference and Student-Newman-Keuls were used for post hoc ANOVA tests. These procedures may be complementary because Fisher protected least significant difference is liberal and sensitive to a Type 1 error but Student-Newman-Keuls test is sensitive to a Type 2 error rather than a Type 1 error (37, 38).

RESULTS

Experiment 1: Neuronal RNA Oxidation in Human Brain Aging

Immunoreactivity for 8OHG was virtually undetectable in subjects younger than 40 years (Fig. 1A), but there was faint or moderate immunoreactivity in neuronal cytoplasm in clinically normal, presenile, and senile subjects (>40 years; Figs. 1B, C). To investigate whether the 1F7 antibody immunoreactivity was derived from oxidized RNA or oxidized DNA or both, we performed nuclease pretreatment before the immunostaining. Immunoreactivity in the sections of the presenile and senile subjects was diminished greatly by RNase pretreatment (Figs. 1F, G) but not by DNase pretreatment (Figs. 1D, E). We also demonstrated this in sections of AD and dementia with Lewy bodies in brains (4, 6, 12). These data indicate that RNA is a major site of nucleic acid oxidation in presenile and senile individuals without dementia as well as in patients with these age-associated neurodegenerative disorders.

Relative scale measurements of the 8OHG immunoreactivity demonstrated an age-associated increase in intensity of neuronal 8OHG immunoreactivity. The relative density of neuronal 8OHG immunoreactivity increased significantly in



the hippocampal subiculum and in the inferior temporal/occipitotemporal gyrus with age (Figs. 1H, I). These results cannot be explained by neuronal shrinkage because the average neuronal cell profile area was not significantly changed during aging ($p = 0.76$ in the hippocampal subiculum and $p = 0.40$ in the inferior temporal/occipitotemporal gyrus by linear regression analysis between age and neuronal cell size). Levels of relative 8OHG immunoreactivity were not related to PMI ($p = 0.13$ in the hippocampal subiculum and $p = 0.65$ in the inferior temporal/occipitotemporal gyrus by linear regression analysis). Furthermore, we found similar average values of relative 8OHG immunoreactivity in subjects who died of accident (5.1), heart failure (6.3), internal malignancy (7.2), rupture of aneurysm (3.9), and others (5.2) in the hippocampal subiculum ($p = 0.45$ by ANOVA); and heart failure (5.3), internal malignancy (5.0), leukemia (5.3), and others (4.6) in the inferior temporal/occipitotemporal gyrus ($p = 0.95$ by ANOVA).

Experiment 2: Neuronal RNA Oxidation in Preclinical AD and MCI

There were no significant differences in age ($p = 0.19$) or PMI ($p = 0.56$) among the 4 categories of subjects by ANOVA (Table). In all 10 subjects classified as having mild AD and MCI, as well as one 95-year-old control subject, neurons with marked cytoplasmic 8OHG immunoreactivity were widely distributed in the temporal cortex, whereas neurons in the same region in the 4 subjects with preclinical AD and the remaining 4 control subjects showed significantly lower levels of 8OHG immunoreactivity (Figs. 2, A–D). In all subjects, Purkinje cells of the cerebellum contained insignificant 8OHG levels (Figs. 2, E–H). Relative 8OHG immunostaining intensities of temporal cortex pyramidal neurons in the mild AD and MCI groups were significantly higher than the preclinical AD and control groups (Fig. 2I). Interestingly, levels of 8OHG immunostaining intensities were higher in the MCI group than in the mild AD group, although not significantly (Fig. 2I). No significant differences among the 4 groups were detected in relative 8OHG immunostaining intensities of cerebellar Purkinje cells (Fig. 2J).

Figure 3 is a plot of relative neuronal 8OHG immunostaining intensities in the temporal cortex as a function of age in 58 subjects (38 subjects from Experiment 1 [Group 2] and 20 subjects from Experiment 2). This shows a distinct range of the relative neuronal 8OHG of the MCI and mild AD from the control subjects with intact cognitive function and subjects with preclinical AD. There was an exceptional control subject (marked by an arrow) with a relative 8OHG level comparable to that of MCI and mild AD subjects; this patient died of renal failure at the age of 95.

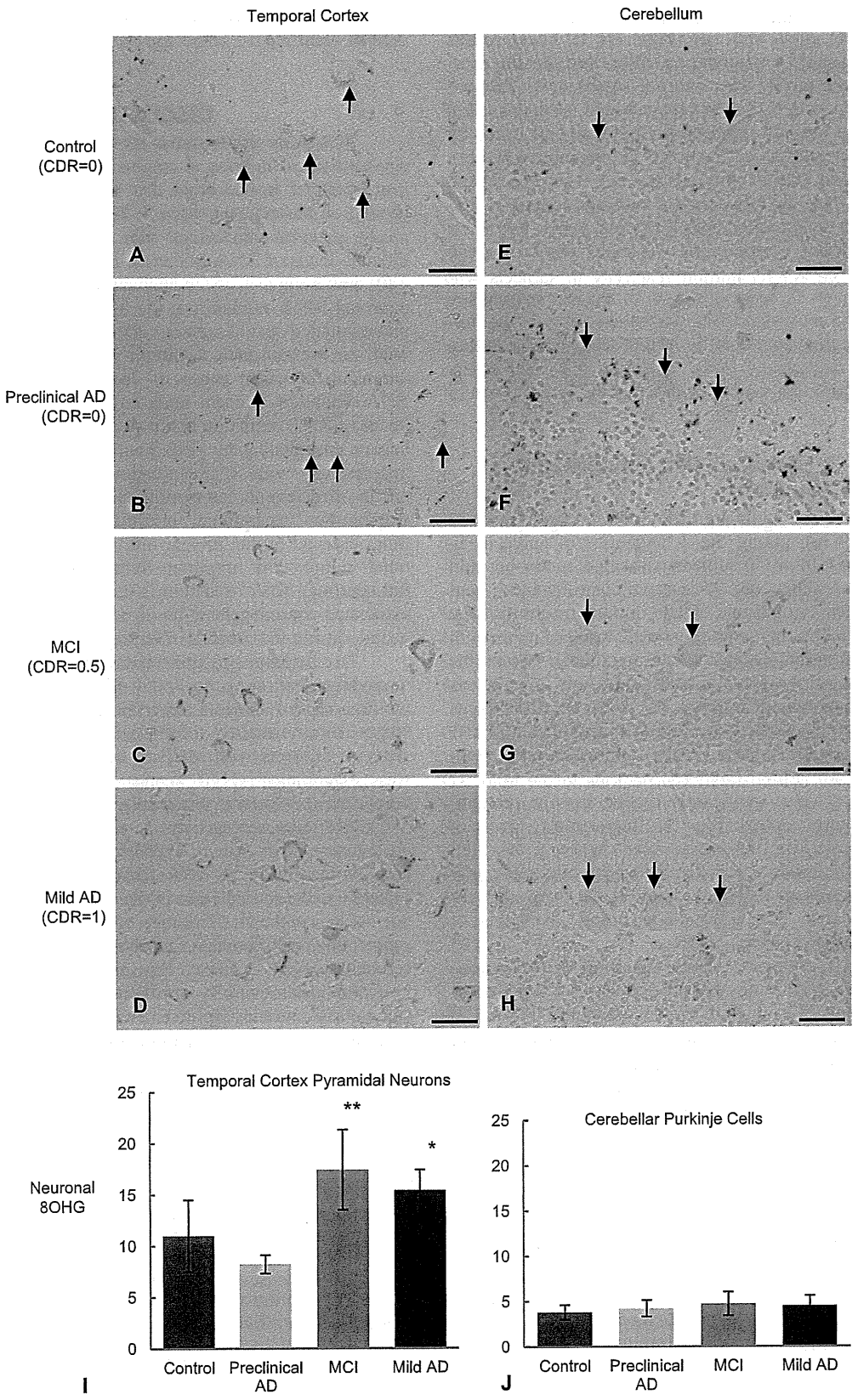
DISCUSSION

This study demonstrates for the first time that there is an age-associated increase in oxidative damage to RNA in neuronal cells of human brain that may be an important background of neurodegeneration in the process of aging. Several recent experimental studies with aging rodents showed significant levels of RNA oxidation in liver (39), skeletal muscle (40), and brain (14, 15) in aged animals. One of them showed neuronal RNA oxidation in the hippocampus of old animals that paralleled spatial memory deficit (14), which is consistent with our present data. Although we found no significant relationship between causes of death and levels of neuronal RNA oxidation, a recent study reported significantly elevated levels of RNA oxidation in the cerebral cortex of patients with hepatic encephalopathy (41). Such an association of metabolic insufficiency with RNA oxidation in brain and a small capacity of metabolic compensation due to advanced age might explain the "exceptional" elevation in neuronal RNA oxidation observed in the 95-year-old control subject who died of renal failure. One important remaining question is whether the neuronal RNA oxidation is a crucial background of age-associated neurodegeneration or is merely a phenomenon that occurs during the process of aging.

To elucidate a possible relationship of oxidized RNA with neurodegeneration at the initial phase of functional decline, we investigated subjects representing clinical and pathologic stages from normality to mild dementia. As in our previous study of subjects with MCI in the hippocampus (23), there was a significant level of neuronal RNA oxidation in the temporal cortex. Selective vulnerability was evident by comparison of 8OHG immunoreactivity in the temporal cortex pyramidal neurons with that in cerebellar Purkinje cells. The neuronal vulnerability in RNA damage is consistent with the selective iron accumulation in the cerebral cortex neurons but not in the cerebellar Purkinje cells in MCI brains, whereas some glial cells contain a considerable level of iron in the cerebellum (42). Because abnormalities in mitochondria and iron homeostasis likely contribute to damage cellular RNA as a source and an amplifier of reactive oxygen species (3), further investigations on mitochondrial dysfunction in MCI may provide a particular key in elucidating the pathogenesis of the onset of cognitive decline.

This study extends the categories of subjects to preclinical cases who exhibit full features of AD brain pathology

FIGURE 1. Neuronal RNA oxidation in the aging human brain assessed in situ using 1F7 mouse monoclonal antibody against 8-hydroxyguanosine (8OHG) in hippocampi of individuals without a history of cognitive impairment. **(A–C)** Immunoreactivity in a young subject (17 years old) **(A)** is virtually undetectable; neurons of a presenile subject (46 years old) **(B)** and a senile subject (77 years old) **(C)** show faint or modest immunoreactivity. Scale bars = 100 μm. **(D–G)** RNA origin of the immunoreactivity is supported by pretreatment of sections with from an 81-year-old subject with nucleases. 8OHG immunoreactivity in hippocampal sections **(D, F)** is almost unchanged by treatment with RNase-free DNase **(E)**; in contrast, it is greatly diminished by treatment with DNase-free RNase **(G)**. **D**, **E**, **F**, and **G**, respectively, are adjacent serial sections. Scale bars = 50 μm. Asterisk (*) indicates landmark blood vessel. **(H, I)** Relative 8OHG immunostaining intensity measurements using a computer-assisted image analysis and linear regression analysis show a significant correlation between age and relative levels of neuronal 8OHG (*y* axis: arbitrary units) in the hippocampal subiculum ($r = 0.55$, $p < 0.01$) **(H)** and in the inferior temporal/occipitotemporal gyrus ($r = 0.47$, $p < 0.01$) **(I)**.



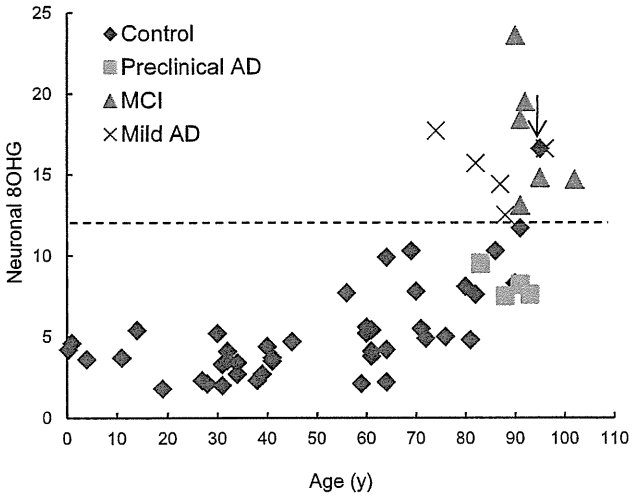


FIGURE 3. Overview of changes in relative levels of neuronal 8-hydroxyguanosine (8OHG) immunoreactivity (*y* axis: arbitrary units) as a function of age in the temporal cortex of all subjects from Experiments 1 and 2. Subjects with mild cognitive impairment (MCI) and mild Alzheimer disease (AD) show a distinct range of relative 8OHG levels (the range above the dotted line) from those subjects with intact cognitive functions, including control subjects and subjects with preclinical AD (the range below the dotted line). Exceptionally, only a 95-year-old control subject (↓) who had intact cognitive function and pathologically had only diffuse plaques of amyloid β showed a relative 8OHG level within the upper range occupied by subjects with MCI and mild AD.

but no or few signs of cognitive decline. We found no significant difference in levels of neuronal RNA oxidation in preclinical cases compared to age-matched controls; this result is consistent with previous results of no significant changes in levels of lipid peroxidation and oxidative protein modification in the cerebral neocortex of preclinical AD (25–27). However, these observations are inconsistent with several previous findings, such as elevation of iron content in brains of preclinical AD (42), elevation of a lipid peroxidation product, F_2-isoprostane, in the cerebrospinal fluid of preclinical familial AD (43), and temporal primacy of neuronal RNA oxidation to amyloid β (Aβ) deposition in brains in an aging series of subjects with Down syndrome (36). Nevertheless, the present data support early involvement of oxidative stress in the transition of normal to cognitive impairment. The National Institute on Aging and the Alzheimer's Association workgroup now accept that, for defined purposes, preclinical AD consisting of approximately 40% of elderly without dementia at the mean age of 84 years (44) is a stage of AD progression (45). Indeed, preclinical AD might represent a compensatory period in which the brain is capable of maintaining cellular vitality and minimizing oxidative stress and, consequently, preserving cognitive function. It is speculated that certain coping mechanisms against formation and/or propagation of free radicals are still intact at the preclinical phase in the cognitively normal elderly; such compensatory mechanism might be compromised even in young adulthood in cases of preclinical familial AD and Down syndrome due to their genetic basis (6, 36).

We previously reported the relationship between levels of extraneuronal and intraneuronal Aβ burden and neuronal oxidative damage to RNA. The results were contrary to the common notion that levels of Aβ burden should parallel each stage of neurodegeneration. Indeed, there was an inverse relationship between levels of extraneuronal Aβ deposition and neuronal oxidative damage to RNA in subjects with sporadic AD (5), familial AD (6), and Down syndrome (36) but no significant relationship in controls (5). Recently, we showed that there was also an inverse relationship between the levels of intraneuronal Aβ42 immunostaining intensity and neuronal oxidative damage to RNA in AD (7). Moreover, we found more prominent oxidative RNA damage in neurons without NFTs or granulovacuolar degeneration versus neurons with NFTs or granulovacuolar degeneration in AD (5, 46). These unexpected observations enabled us to hypothesize that the development of AD-related pathology might represent a compensatory response to neuronal oxidative insults (7, 46, 47). In this context it would be very interesting to learn whether there is a specific relationship between Aβ, NFTs, or granulovacuolar degeneration burden and neuronal oxidative damage in the transitional stages from normal aging to the onset of AD.

Recent studies have indicated that RNA oxidation has detrimental effects on cellular function whether the damaged RNA species are coding for proteins (messenger RNA) or performing translation (ribosomal RNA and transfer

FIGURE 2. Neuronal RNA oxidation in subjects with preclinical Alzheimer disease (AD), mild cognitive impairment (MCI), and mild AD (Experiment 2) assessed using the 1F7 antibody against 8-hydroxyguanosine (8OHG) in the temporal cortex and cerebellum. **(A–D)** Neurons in the temporal cortex of a control subject (90 years old; CDR = 0) **(A)** and a subject with preclinical AD (91 years old; CDR = 0) **(B)** show only faint or moderate 8OHG immunoreactivity (upward pointing arrows); neurons in the temporal cortex of a subject with MCI (92 years old; CDR = 0.5) **(C)** and a subject with mild AD (88 years old; CDR = 1) **(D)** show prominent immunoreactivity that is predominantly in the cytoplasm. **(E–H)** 8OHG immunoreactivity in the cerebellum from the same subject as shown in **A**, **B**, **C**, and **D**, respectively. In all subjects, 8OHG immunoreactivity is virtually undetectable in cerebellar Purkinje cells (downward pointing arrows). Scale bars = 50 μm. **(I, J)** Relative 8OHG immunoreactivity measurements and ANOVA with post hoc Fisher protected least significant difference demonstrate that there are significant increases in relative levels of 8OHG (*y* axis: arbitrary units) in pyramidal neurons of the temporal cortex **(I)**, but not in the cerebellar Purkinje cells **(J)**, in the MCI subject versus control subjects (**, $p < 0.01$) or preclinical AD subjects (**, $p < 0.01$), as well as in the subjects with mild AD versus the control subjects (*, $p < 0.05$) or the subjects with preclinical AD (*, $p < 0.05$). These differences in the multiple comparisons were significant also with post hoc Student-Newman-Keuls test ($p < 0.05$ in all comparisons). Subjects with MCI show higher levels of 8OHG than those with mild AD, although the differences are not significant. Data are mean ± SD. CDR, Clinical Dementia Rating.

RNA) (8, 10, 22, 48, 49). Indeed, levels of neuronal RNA oxidation parallel spatial memory deficits in aging animals, and age-associated oxidative RNA damage and memory deficits can be reduced by antioxidants or mitochondrial metabolites (14). Therefore, oxidative RNA damage in vulnerable neurons is a promising candidate of targets for prevention and treatment of AD from prodromal to early stages of the disease.

In summary, the present results indicate that 1) a modest level of oxidative RNA damage in cerebral neurons occurs during the process of aging and 2) a prominent level of oxidative RNA damage in vulnerable neurons corresponds with the earliest stage of cognitive decline in the transition from cognitively normal aging to AD. Further investigations aimed at the cellular consequences of oxidative RNA damage and compensatory mechanisms might provide insights into the process of aging and the pathogenesis of age-associated neurodegeneration. Such approaches may provide novel strategies for prevention and treatment of AD.

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認知症学 下

—その解明と治療の最新知見—

III. 臨　床　編

血管性認知症とその類縁疾患　各論

Vascular cognitive impairment

布村明彦　　玉置寿男

血管性認知症とその類縁疾患　各論

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Key words：血管性認知症，血管性認知障害，軽度認知障害，vascular cognitive impairment-no dementia，vascular mild cognitive impairment

はじめに

血管性認知障害(vascular cognitive impairment: VCI)は脳血管障害(cerebrovascular disease: CVD)に起因あるいは関連する認知機能障害のすべてを指す[1]．すなわちVCIは，血管性軽度認知障害(vascular mild cognitive impairment: vascular MCI)や血管性認知症(vascular dementia: VaD)のみならず，CVDに基づく失語症などの単独認知ドメインの障害やCVDを伴うアルツハイマー病(Alzheimer's disease: AD)も包含する極めて広範な概念である[2]．

本稿では，vascular MCIに相当するVCI-no dementia(VCI-ND)を中心に解説する．

1. VCI概念が提唱された背景

a. AD with CVDへの注目

認知症の概念および臨床診断において，変性性か虚血性か，あるいはADかVaDかという二分法が長い間支配的であった．しかし近年，ADがCVDと共通の血管性危険因子を有することや，脳病理学的にもADの病理所見とCVDが共存する病態が認められることが明らかにされ，二分法的解釈では臨床診断を困難にし，病態解明や治療法開発にも停滞を招くと考えられるようになった[3]．現在では，CVDを有する認知症は必ずしもVaDではないという認識が一般的になり，CVDを伴うAD(AD with CVD)という概念も広く受け容れられている．AD with CVDも包含する概念としてVCIが提唱された背景に，このような認知症概念の変遷がある．

b. Vascular MCIへの注目

VaDの診断基準として臨床研究で頻用されるNINDS-AIREN基準では，特異度は高いが感度が低いという問題がある．その理由としてAlzheimerization Bias，すなわちADに準じた記憶障害を必須条件とする評価基準であるためVaDで認められる注意や遂行機能の障害が的確に検出されないことが挙げられている[2]．したがって，既存のVaD診断基準では記憶障害が目立たない，軽症の認知機能障害例の多くが見逃される危険性がある．近年，認知症の前駆症としてMCI概念が普及し，MCI段階から予防・治療介入を行う必要性が強調されるようになった．vascular MCIも包含する概念としてVCIが提唱された背景に，このような早期診断を重視する動向がある[4]．

2. VCIの定義

a. 広義VCI

VCIは，vascular MCIやVaDのみならず，CVDに基づく失語症など単独認知ドメインの

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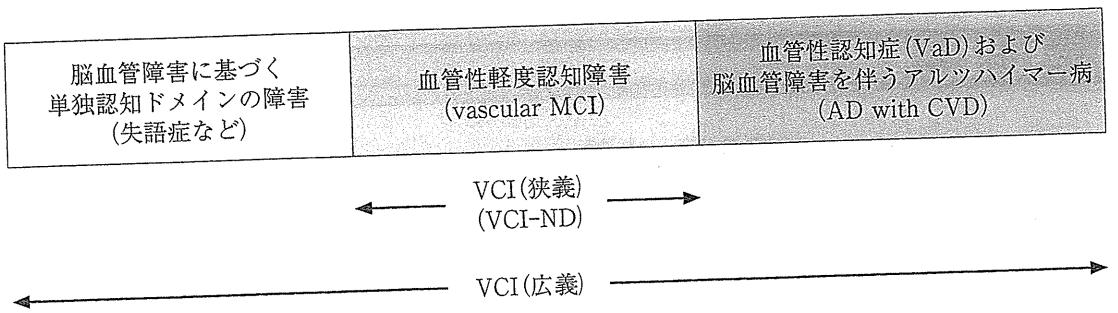

図1 血管性認知障害（vascular cognitive impairment: VCI）の概念

AD: Alzheimer's disease, CVD: cerebrovascular disease, MCI: mild cognitive impairment, VaD: vascular dementia, VCI-ND: vascular cognitive impairment-no dementia.

障害やAD with CVDも包含する極めて広範な概念である（**図1**，広義VCI）[2]．VaD一つを取り上げてみても，その臨床病型が，①多発梗塞性認知症（≒皮質性VaD），②戦略的な部位の単一梗塞による認知症，③小血管病変性認知症（≒皮質下VaD；多発性ラクナ梗塞性認知症とBinswanger病を含む），④低灌流性認知症，⑤出血性認知症など多岐にわたり，VCIの基盤になるCVDは多様である．

b. 狭義VCI（VCI-ND）

広義VCIの概念はあまりに広範であり，CVDに関連する認知機能障害のうち，認知症には至らない軽症のもの（VCI-ND），すなわちvascular MCIのみを指してVCIと呼ぶべきであるという考え方もある（**図1**，狭義VCI）[2]．

3. VCIの発生頻度と予後

Inglesらの報告[5]では，65歳以上の健常高齢者を5年間追跡したところ，12％に広義VCIが認められ，内訳は44％がVCI-ND，32％がVaD，24％がAD with CVDであった．広義VCI発症群は，AD発症群や正常認知機能維持群と比較してベースラインの抽象的思考が低得点だった[5]．

Meyerら[6]による健常高齢者（平均年齢68歳）の追跡研究では，平均3.7年の追跡期間中に1/4がMCIを発症した．MCIの約半数はADに，約2割はVaDに進行し，約3割はstable MCIであった．追跡期間中に健常高齢者の約5％がMCIを経てVaDを発症し，その2/3は小血管病変性認知症（皮質下VaD）であった．他方，MCIを経ずにVaDを発症する場合もあり，その発生頻度は健常高齢者の約4％で，2/3は多発梗塞性認知症か単一梗塞による認知症であった．

VCI-ND（平均79歳）の追跡研究[7]では，5年後に44％の症例がVCI-NDから認知症に進展し，内訳は43％がVaD，35％がAD，13％がAD with CVD，9％が未分類であった．VCI-NDの時点における記憶課題とカテゴリー流暢性課題での低得点が認知症への進展と関連していた[7]．

VCI-NDでは経過中に認知機能が正常化する例がある[8]．一方で死亡率が高いことも指摘され，VCI-ND（平均79歳）は5年間に52％が死亡した[8]．vascular MCI（平均78歳）の追跡研究[9]でも，40カ月間に50％が死亡し，生存例にも認知機能や運動・排泄機能の悪化例が多く，施設入所者が多かった．

4. VCIの診断

a. 診断基準と臨床症状

広義VCIは，多種多様なCVDに起因・関連するうえにAD病理の関与も想定しているものであり，統一的な診断基準を作成することは困難と考えられている[4]．したがって現時点でVCI診断基準は存在しておらず[3]，国際ワーキンググループによる今後のVCI研究指針が示されているにすぎない[1]．

広義VCIでは，皮質下性の認知機能障害である精神運動の緩慢化や遂行機能障害が特徴的とされる[10]．一方，ADに比べてVCIではうつ症状

が高頻度で進行性であるが，遂行機能障害の頻度と進行度はADとVCIの間に有意差はないという報告もある[11].

狭義のVCI(vascular MCIあるいはVCI-ND)は，CVDを基盤として認知機能の単独ドメイン，すなわち，記憶，言語，注意，遂行機能，視空間認知などのいずれか，あるいはこれらの複合ドメインの障害を認めるが，認知症には進展していない状態(日常生活機能はほぼ正常)を指す．VCI-NDの特徴として，精神運動の緩慢化，注意および遂行機能の障害が血管病変を伴わないMCIに比べて顕著であることが挙げられる[12]．神経症状ではパーキンソニズム(特に歩行障害や姿勢反射障害)が高頻度に認められる[9]．しかし，これらの特徴はVCI-NDに特異的ではない[4].

b. VCI-NDのスクリーニング検査

追跡研究でADあるいはVaDを発症した症例のMCI時点でのmini-mental state examination(MMSE)下位項目スコア(記憶，見当識，注意，言語，および視空間認知)を比較すると，2群間に有意差は認められなかった[6].

臨床的に簡便に施行でき，MCIの診断に有用なスクリーニング検査として，Nasreddineらが2005年に開発したMontreal Cognitive Assessment(MoCA)が注目されており，日本語版(MoCA-J)[13]も2010年に作成されている．認知症のスクリーニング検査として開発されたMMSEとは異なり，MoCAは軽度認知機能低下のスクリーニング検査として開発され，MMSEに比べて注意と視空間認知の評価が詳細で高配点であり，抽象的思考と遂行機能も評価される(**表1**)．Pendleburyら[14]は，一過性虚血発作あるいは脳卒中後にMMSEが27点以上であった症例の58％でMoCAは25点以下とMCIレベルを示唆し，MMSEでは検出されないVCI-NDがMoCAによって感度良く検出されたと報告している．MoCAのVCI-ND診断上の有用性について今後の検討が期待される.

c. VCI-NDの画像診断

VCI-NDは多種多様なCVDに関連することから，画像所見も様々なパターンを呈する．疫学研究から皮質下の小血管病変が認知機能低下や認知症発症の危険因子であることが知られているが，個々の症例において脳画像上の虚血性病変がどの程度臨床症状に関連しているかを判定することは困難な場合が多い[10].

Meyerら[15]は，健常高齢者ならびに各種MCIおよび各種認知症に進展した例の脳MRI所見について比較検討した．その結果をvascular MCIを中心に**表2**にまとめた．健常高齢者と比較してvascular MCIでは皮質萎縮，中心性萎縮，および皮質下白質病変が高度である．変性性認知症に前駆するMCIと比較すると，vascular MCIでは内側側頭葉などの萎縮性変化は軽度だが，皮質下白質の虚血性変化が高度である．また，皮質下白質の虚血性変化と脳前方部の中心性萎縮の進行がvascular MCIからVaDへの進展に関連することが示唆された.

5. VCI-NDに対する治療介入

VCI-NDに対する治療介入の最大の目的は認知症への進展予防であるが，VCI-NDを対象に行われた大規模な介入試験は見当たらない.

多数の観察的疫学研究から，中年期の高血圧および糖尿病は認知機能障害および認知症の危険因子と考えられている．また，脂質異常症や心房細動も認知症の危険因子に数えられている[16]．これら血管性危険因子に対する治療薬がVCI発症予防やVCI-NDから認知症への進展予防に役立つ可能性がある．降圧療法に関しては複数のランダム化比較試験(RCT)によって，認知機能低下の抑制効果や認知症発症率の低減効果が報告されており，中年期の降圧療法は認知機能障害に対する予防として推奨される[17]．他方，観察的疫学研究の結果から期待されたスタチン投与，II型糖尿病の治療，あるいはアスピリン投与による認知機能低下の抑制効果は，RCTでは否定的な結果であった[17,18].

頸動脈の高度狭窄病変を有するvascular MCIに対しては，内膜剥離術によって60％の症例が正常認知機能に復したという報告がある[19].

観察的疫学研究から，女性では活発な運動習慣がVCI-NDのリスク低下に関連していたと

表1 Mini-mental state examination（MMSE）とMontreal Cognitive Assessment（MoCA）の比較

評価項目		MMSE配点	MoCA配点
見当識	時間の見当識	5	4
	場所の見当識	5	2
	計	10	6
記憶	即時想起（単語リストの復唱）（MMSE: 3単語；MoCA: 5単語）	3	0
	遅延再生	3	5
	計	6	5
注意	連続減算（シリアル7）	5	3
	5文字の逆唱（シリアル7不能の場合のみ）	(5)	—
	数唱　5桁順唱	—	1
	3桁逆唱	—	1
	ビジランス（‘あ’で手を叩き，‘あ’以外の音で叩かない）	—	1
	計	5	6
言語/従命動作	文章の復唱	1	2
	呼称・命名（MMSE: 2物品；MoCA: 3動物の絵）	2	3
	文章の作成（自発書字）	1	—
	口頭指示に従う動作（3段階）	3	—
	書字指示に従う動作（閉眼）	1	—
	計	8	5
抽象的思考	類似問題（‘電車’と‘自転車’＝乗り物，など）	0	2
	計	0	2
遂行機能	語流暢性（‘か’で始まる単語の列挙）	—	1
	trail making test（B）	—	1
	計	0	2
視空間認知/構成	図形模写（MMSE: 5角形の交差図；MoCA: 立方体図）	1	1
	時計描画（輪郭，数字，針）	—	3
	計	1	4
合計得点（満点）		30	30*
カットオフ値	認知症/MCIあるいは健常	23/24	—
	MCI/健常	—	25/26

*教育年数12年以下の場合には1点加点.

報告されている[20]．VCI-NDに対する非薬理学的介入効果の検証は今後の重要な課題である．

おわりに

VCIは，変性と虚血の垣根を越えて認知機能

表2 Vascular MCI(VCI-ND)の脳MRI所見(文献[15]より引用)
—健常高齢者，他のMCIサブタイプ，および血管性認知症(VaD)との比較—

脳MRI上の変化	vs健常高齢者	vs AD-MCI	vs PDD/DLB-MCI	vs VaD
前頭葉皮質萎縮	↑			
側頭葉皮質萎縮	↑			
頭頂葉皮質萎縮	↑			
後頭葉皮質萎縮	↑			
第三脳室拡大	↑		↓	
側脳室前角拡大	↑			↓
側脳室下角拡大		↓		
海馬萎縮		↓		
嗅内皮質萎縮		↓	↓	
白質ラクナ梗塞	↑	↑	↑	
leukoaraiosis	↑	↑		↓

↑：vascular MCIの方が比較する群よりも高度，↓：vascular MCIの方が比較する群よりも軽度，AD-MCI：アルツハイマー病に前駆するMCI，PDD/DLB-MCI：認知症を伴うパーキンソン病/レビー小体型認知症に前駆するMCI.

障害を初期段階からとらえるうえで有用な概念であり，認知症の早期介入方法を開発するうえでも今後益々重要性が認識されるだろう．特にVCI-NDの中核をなす皮質下血管障害を伴うMCIは，生命予後および機能予後ともに悪く，早期介入の必要性が高いことに注意すべきである．

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連載

老年期精神医学⑦

アルツハイマー病根本治療薬の開発*

布村明彦**

Key Words：Alzheimer's disease, amyloid cascade hypothesis, disease-modifying drugs, MCI, preclinical stage

はじめに

アルツハイマー病(Alzheimer's disease；AD)患者数は現在，全世界で3,500万人に達すると推計されており，2050年には1億人を突破すると予想されている．この現在進行中の"AD epidemic"に対して有効な対策がとられないならば，個人，経済，および社会が被る損失は計り知れないと警告されている[1]．

本年，わが国において従来認可されていたアセチルコリン・エステラーゼ阻害薬donepezilに加えて，同様の薬理作用を持つgalantamineおよびrivastigmine，ならびにNMDA受容体拮抗薬memantineがAD中核症状治療薬として承認された．これらの治療薬は，神経変性の結果生じたコリン作動性あるいはグルタミン酸作動性の神経伝達機能障害を改善させる効果が主体であり，症候改善薬(symptomatic drugs)に位置づけられる．各治療薬の特徴を理解して適切に使用することはADの臨床上重要であり，今後わが国での使用経験の蓄積によって治療薬選択の最適化が進むことが期待される．

他方，ADの神経変性過程に直接的に作用して神経細胞死を抑制する治療法の確立を目指し，AD根本治療薬(疾患修飾薬；disease-modifying drugs)の開発が進められているが，現在までにどのような成果が得られているのであろうか．アミロイドβタンパク(Aβ)を標的にしたAD根本治療薬候補の臨床試験はすでに3剤が第III相まで進行したが，いずれも不成功に終わった[1)2)]．バイオマーカー研究の進歩を受け，根本治療薬の対象は発症後早期からさらに遡って前臨床期(preclinical stage)まで視野に入ってきた[1)2)]．本稿では，AD根本治療薬開発の近年の動向を概観し，今後の方向性について考察する．

アミロイド・カスケード仮説

AD脳を特徴づける神経病理学的変化として，老人斑と神経原線維変化があげられる．これら古典的病理構造物に関して1980年代以降に飛躍的な研究の進歩があり，老人斑は凝集したAβから構成され，神経原線維変化は過剰にリン酸化されて凝集したタウタンパクから構成されていることが明らかになった(図1)．続いてAβの前駆体タンパク(amyloid precursor protein；APP)がクローニングされ，家族性ADの家系において*APP*遺伝子の変異が見出された．また，APPをコードする21番染色体のトリソミーであるダウン症候群の脳ではADに合致する病理変化が認められるが，年代順の検討から老人斑が神経原線維変化に先行して認められることが知られていた．これらの知見から，Aβの凝集・沈着こそがADの原因であり，その下流で神経原線維変化や神経細胞死が生じるとする「アミロイド・カスケード

* Geriatric Psychiatry⑦—Development of disease-modifying drugs for Alzheimer's disease.

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