タゾンを投与し，メタボリックシンドロームの各種コンポーネントに及ぼす影響を検討したところ，インスリン感受性の増加と耐糖能の改善に加え，血清トリグリセリド値，総コレステロール値の低下ならびにHDLコレステロール値の増加がみられた[12]．またピオグリタゾンは，これらの患者において血清アディポネクチン値の上昇とTNF-α値の低下(**図2-b**)をもたらすことも明らかとなった[11]．

4. 今後の展望

最近著者らは，画像上内臓脂肪の蓄積は認めないが軽度のインスリン抵抗性を示すWerner症候群の若年例(29歳，女性)に遭遇した．最終結論は今後の経過観察に委ねられるが，Werner症候群にみられる複合代謝異常の一次的要因はインスリン感受性の低下にあり，内臓脂肪の増加は代償的高インスリン血症に伴う二次的な変化であることが推察される．WRNヘリカーゼの変異がどのような機序でインスリン抵抗性をもたらすかは未解明だが，実験的観察事実としてはWerner症候群患者の線維芽細胞において，グルコーストランスポーターGLUT1の細胞膜への移行の障害が報告されている[13]．

内臓脂肪量の増加とインスリン感受性の低下は，一般的な加齢においても観察される変化である[14]．近年，Werner症候群と並ぶ代表的な早老症であり，10歳代で心筋梗塞を発症するHutchinson-Gilford症候群の原因がlamin A遺伝子の変異にあると特定された[15]．lamin Aの変異は，インスリン抵抗性と関連の深い部分的脂肪萎縮症(partial lypodystrophy)の原因としても知られていることから，早老症発生の分子機序に関する研究は，老化-インスリン抵抗性-動脈硬化を結ぶ新たな手がかりを与えてくれることが期待される．

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A patient with Werner syndrome and adiponectin gene mutation

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Abstract

Werner syndrome is a premature aging disease characterized by genomic instability and increased cancer risk. Here, we report a 45-year-old diabetic man as the first Werner syndrome patient found to have an adiponectin gene mutation. Showing graying and loss of hair, skin atrophy, and juvenile cataract, he was diagnosed with Werner syndrome type 4 by molecular analysis. His serum adiponectin concentration was low. In the globular domain of the adiponectin gene, I164T in exon 3 was detected. When we examined effects of pioglitazone (15 mg/day) on serum adiponectin multimer and monomer concentrations using selective assays, the patient's relative percentage increased in adiponectin concentration was almost same as that in the 18 diabetic patients without an adiponectin mutation, but the absolute adiponectin concentration was half of those seen in diabetic patients treated with the same pioglitazone dose who had no adiponectin mutation. The response suggested that pioglitazone treatment might help to prevent future Werner syndrome-related acceleration of atherosclerosis. Present and further clinical relevant to atherosclerosis in this patient should be imformative concerning the pathogenesis and treatment of atherosclerosis in the presence of hypoadiponectinemia and insulin resistance.

Keywords: Werner syndrome; Adiponectin mutation; Diabetes mellitus; Hypoadiponectinemia; Thiazolidine therapy

1. Introduction

Werner syndrome is an autosomal recessive hereditary disease characterized by premature aging, genomic instability, and accelerated atherosclerosis, and increased cancer risk [1,2]. The defective gene product in Werner syndrome belongs to the ReqQ family of DNA helicases [3]. Here, we report the first patient with Werner syndrome found to have an adiponectin gene mutation as well. We examined changes in adiponectin secretion in response to pioglitazone therapy.

2. Case presentation

A 45-year-old man was diagnosed with diabetes when cataract developed at the age of 25 years. He did not seek further treatment until he was 39 years old, when he was admitted to another hospital. There he was given insulin and was noted to have abdominal fat accumulation. He was referred to our hospital in April 2004.

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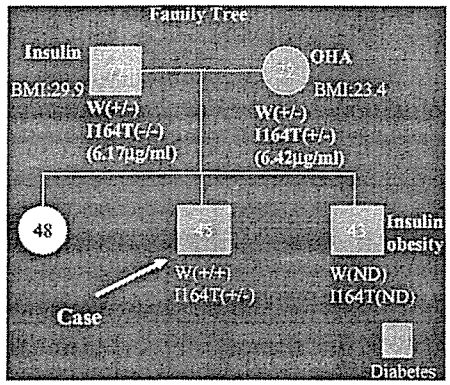

Fig. 1. The patient's father, mother, and uncle had diabetes; the father and uncle were treated with insulin, and the mother with oral hypoglycemic agents. Both parents were heterozygous for Werner syndrome type 4, and heterozygosity for the adiponectin gene mutation I164T was identified in the mother. Values shown are serum adiponectin concentrations (μg/ml).

The patient's father and uncle had diabetes; recently, his mother also had been diagnosed with diabetes. Fig. 1 shows the patient's family tree. His parents both were found to be heterozygous for the Werner mutation, while his mother was heterozygous for an I164T mutation in the adiponectin gene. No consanguinity was reported.

Height was 151.8 cm and weight was 38 kg. Blood pressure was 158/80 mmHg and the pulse was regular with a rate of 92 min^{-1}. The patient injected insulin before each meal (Penfil R 6U) and before sleep (Penfil N 6U). Hemoglobin (Hb) A1c was 6.7%; total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were 208, 59, and 190 mg/dl, respectively. Urinary albumin excretion was 26.5 mg/g creatinine. The serum C-peptide concentration was 6.61 ng/ml with a simultaneous plasma glucose concentration of 156 mg/dl, suggesting that insulin secretory capacity was preserved and implying that insulin resistance was likely. The patient showed graying and loss of hair, skin atrophy, and juvenile cataract. We diagnosed him with Werner syndrome type 4 according to molecular analysis [4].

Yokote et al. [5], previously, reported serum adiponectin concentrations to be decreased in Werner syndrome (mean 3.1 μg/ml); our patient's serum adiponectin concentration was particularly low (2.24 μg/ml; to adiponectin monomer assay kit, Otsuka, Tokyo, Japan). When we sequenced the adiponectin gene, heterozygous mutation representing I164T in exon 3 was seen in the globular domain, as was demonstrated in his mother. This mutation has been reported to be atherogenic and to promote insulin resistance, leading to ischemic heart disease [6]. As the adiponectin and Werner genes are located on chromosome 3 and 8, respectively. We concluded that the two mutations were associated coincidentally.

To evaluate vascular atherosclerosis, carotid intima media thickness (IMT) was examined ultrasonographically. While this was only 0.6 mm, calcified plaques 2 mm in thickness were observed in right and left carotid arteries.

We next examined the effects of pioglitazone (15 mg/day) on adiponectin concentrations in the patient using separate adiponectin assay kits to detect the total monomers (Otsuka) and multimeric forms (Fujirebio, Tokyo, Japan). We compared his response to treatment with those in 18 diabetic patients whose adiponectin exon sequences were normal. Responses of serum adiponectin concentrations in the assay for monomers to 15 mg/day of pioglitazone in the other 18 diabetic patients were as follows: 5.68 ± 0.67 μg/ml before pioglitazone, 11.76 ± 1.85 μg/ml (at 1 month), and 11.81 ± 2.20 μg/ml (at 2 months, mean ± S.E.M.). In the Werner patient, the pretreatment adiponectin monomer concentration was 2.32 μg/ml; the1-month value, 6.07 μg/ml; the 2-month value, 5.02 μg/ml (Fig. 2A). Expressed relative to basal concentrations, responses of adiponectin monomer concentrations in

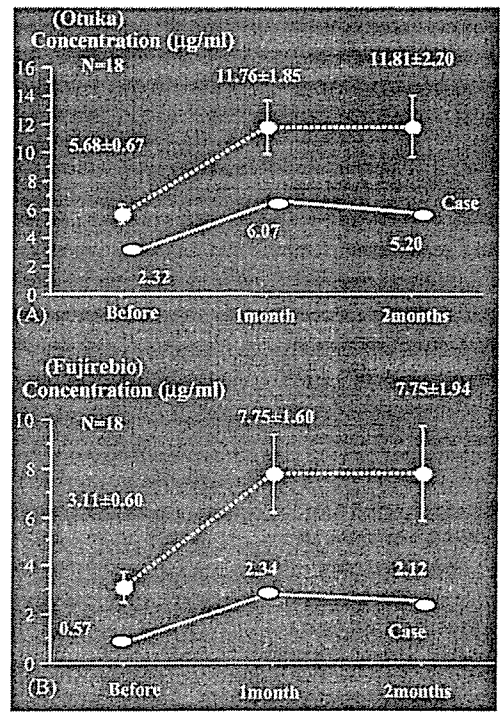

Fig. 2. A: Serum adiponectin concentrations (A, monomer; B, multimer) in response to 15 mg/day of pioglitazone. Data are shown for 18 diabetic patients without an adiponectin gene mutation (means ± S.E.M., broken line) and for the Werner patient (solid line).

the 18 patients with no mutation were 218.5 ± 16.1% (1 month) and 235.7 ± 16.2% (2 months). The Werner patient's relative responses were similar (261.6%, 1 month; 224.1%, 2 months). Serum adiponectin multimer concentrations in response to 15 mg/day of pioglitazone in the diabetic patients without an adiponectin mutation were as follows: 3.11 ± 0.60 μg/ml (pretreatment), 7.75 ± 1.60 μg/ml (1 month), and 7.75 ± 1.94 μg/ml (2 months); in the Werner patient, these, respectively, were 0.57, 2.34, and 2.12 μg/ml (Fig. 2B). For the multimeric form, relative responses in the18 patients were 284.8 ± 25.9% (1 month) and 326.4 ± 35.7% (2 months). In the Werner patient, these, respectively, were 410.5% and 371.9%.

3. Discussion

The adiponectin I164T mutation has been reported to interfere with adiponectin secretion in transfected cultured cells [7,8]. Kadowaki et al. reported that I164T adiponectin could not assemble into trimers, resulting in impaired secretion from the cell [7]. Another study using gel filtration reported that oligomerization was similar to that seen in wild-type adiponectin, but secretion from adipocytes into plasma was disrupted [8]. In our patient's response to pioglitazone, the serum adiponectin concentration was only half that seen in diabetic patients without mutation of the adiponectin gene, suggesting that secretion of mutant adiponectin from adipose tissues into plasma might be disturbed, and with only the wild-type adiponectin responding. The absolute change in serum concentration of adiponectin multimer, measured in response to pioglitazone, was slightly less than that of the monomer in the Werner patient compared with the other 18 diabetic patients, suggesting that processing of mutatnt adiponectin monomer to high-molecular-weight multimer might be compromised.

Here, we first reported a Werner syndrome patient with an additional mutation involving the adiponectin gene. Our study suggested that despite some differences between monomeric and multimeric forms, serum concentrations of both forms of adiponectin could be increased by treatment with thiazolidine derivatives in patients with hypoadiponectinemia resulting from a heterozygous adiponectin gene mutation. These and future data concerning long-term effects on atherosclerosis in this patient may be informative concerning the pathogenesis and treatment of atherosclerosis associated with hypoadiponectinemia and insulin resistance.

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Cognitive Function in the Elderly with Diabetes Mellitus

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Summary **Impaired cognitive function in the elderly with diabetes mellitus has been reported, and many, but not all, studies showed that diabetes mellitus is a risk factor for dementia. Although the mechanism of cognitive dysfunction in elderly diabetes mellitus subjects remains to be elucidated, several lines of evidence indicate that insulin and/or inflammation may be involved.**

***Key Words*: diabetes mellitus, dementia, Alzheimer's disease, insulin, TNF-α**

Introduction

The number of patients with diabetes mellitus (DM), especially type 2 DM, is increasing all over the world, including Japan. DM is an age-related condition, and one-third of the DM patients in Japan are over 60. Although the influence of DM on brain functions has been studied for more than 80 years [*1*], the increase of elderly DM patients has recently caused more interest to be paid to the cognitive functions of these subjects.

Cognitive Decline in DM Elderly

DM-related cognitive decline has been reported in many cross-sectional and in several longitudinal studies [*2–4*]. We studied the cognitive functions of Japanese non-demented elderly DM patients and compared them to age-matched non-DM subjects (Table 1) [*5*]. Subjects with a diagnosis of dementia and/or whose score on the Mini Mental State Examination (MMSE) [*6*] was 23 or lower were excluded, as were those who had a clinical history and/or neurological symptoms of stroke. The cognitive assessment included the MMSE, a Word List recall (immediate and delayed) activity from a subtest of the Alzheimer's Disease Assessment Scale (ADAS) [*7*], a Digit Symbol Test, which is a subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [*8*], and the Stroop Color-Word Test [*9*]. In this study, slight but significant cognitive impairment in elderly DM subjects was found in Japanese DM elderly (Table 2).

Dementia in DM Elderly

Several cross-sectional and longitudinal studies showed that DM is associated with dementia. The Rotterdam study reported that DM is a risk factor for Alzheimer's type dementia [*10*], and several longitudinal data are in agreement with this finding [*11–13*]; however, other studies showed that DM is associated with vascular dementia, not with Alzheimer's type dementia [*14–16*].

The association of AD pathology and DM is also controversial; the Honolulu-Asia Aging study reported that DM subjects had more neuritic plaques and neurofibrillary tangles in the hippocampus [*17*], but in contrast with this, autopsy samples from a Jewish nursing home showed less AD-related pathology in DM subjects [*18*].

Brain Atrophy in DM Elderly

In 1994, a Japanese group reported that elderly DM subjects (6th to 8th decade) had more frequent cerebral atrophy than non-DM subjects [*19*]. We also reported the

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Table 1. Characteristics of participants by diabetes status

Variable	DM subjects	Non-DM subjects	P value
N	69	27	—
Age	71.6 ± 5.6	73.4 ± 6.6	0.164
Gender (% female)	70.4	52.2	0.107
Education (years)	10.4 ± 2.7	11.4 ± 3.0	0.167
Hypertension (%)	52.5	50.0	0.845
Hyperlipidemia (%)	36.5	60.0	0.074
HbA1c (%)	8.0 ± 1.0	5.7 ± 0.4	P<0.01

Data are the mean ± SD unless otherwise indicated.
Student's unpaired *t*-test (age, education, HbA1c) and Kruskal-Wallis analysis (other variables).

Table 2. Performance on measures of cognitive function by diabetes status

Measure	DM subjects	Non-DM subjects	P value
MMSE	27.1 ± 2.2	28.3 ± 1.7	P<0.05
Word List (immediate)	5.7 ± 1.7	6.2 ± 1.7	0.254
(delayed)	7.1 ± 2.2	6.7 ± 2.0	0.364
WAIS-R Digit Symbol	36.3 ± 10.9	43.0 ± 12.1	P<0.05
Stroop Color Word Test	19.2 ± 12.8	15.0 ± 6.7	0.113

Data are the means ± SD, unless otherwise indicated.
A higher score indicates better performance, except in the case of the Stroop Color Word Test.
Student's unpaired *t*-test.
WAIS-R: Wechsler Adult Intelligence Scale-Revised.

Table 3. Values of brain CT measurements in DM and CR subjects

Indices	DM	CR	P value
Evan's ratio	0.264 ± 0.024	0.248 ± 0.029	0.032
i-CM	0.239 ± 0.033	0.230 ± 0.028	0.344
temporal horn (right) (mm)	10.3 ± 4.6	7.9 ± 3.0	0.048*
temporal horn (left) (mm)	10.7 ± 5.8	7.7 ± 3.0	0.046*
maximal width of Sylvian fissure (right) (mm)	24.3 ± 5.1	21.7 ± 5.5	0.091
maximal width of Sylvian fissure (left) (mm)	23.8 ± 4.0	21.7 ± 4.7	0.218

*p<0.05 by ANOVA followed by Scheffe's post hoc test
Indices used to assess brain CT were: a) Evan's Ratio, the maximal width of the frontal horns divided by the maximal internal width of the skull on the same slice level , b) inverse Cella Media Index, the minimum width of the lateral ventricles at the cella media region divided by the maximal internal width of the skull on the same level, c) width of the third ventricle (mm), d) temporal horns, the maximal width of [the] temporal horn tips (mm) on both sides, e) the maximal width of the Sylvian fissure (mm) at the insula on both sides.

widening of the temporal horns on both sides in DM subjects (mean age 71.2), suggesting the atrophy of the hippocampus (Table 3) [*20*], results which were in agreement with those from the Rotterdam study. In this study, MRI volumetric techniques were used to demonstrate the atrophy of the hippocampus and amygdala in elderly DM subjects [*21*]. A large multicenter collaborative study in Europe demonstrated that DM in the elderly (65–75) was associated with cortical brain atrophy, and that a strong interaction between diabetes and hypertension existed [*22*]. A large study in the USA also demonstrated that DM was associated with brain atrophy in a relatively young cohort (mean 62.3) [*23*].

Pathological Mechanism of DM-related Cognitive Decline

Involvement of insulin

The Rotterdam study reported that DM subjects with insulin treatment had a stronger risk for dementia [*10*]. Several studies demonstrated that hyperinsulinemia or insulin resistance is associated with cognitive decline or AD risk [*24, 25*]. Although the mechanism of this association is not clear, several hypotheses have been proposed. High insulin levels may be a reflection of a sedentary life-style, which is associated with a higher risk of dementia [*26*]. Peripheral insulin is transported to the brain across the blood-brain barrier, and insulin receptors exist in the hippocampus and other brain regions [*27*]. Insulin may have some effects on the neurons in the CNS. Recently, insulin degrading enzyme (IDE) has been found to play a role in regulating amyloid beta, a peptide which is closely involved in AD pathology, and insulin may inhibit the activity of the degradation of amyloid beta by IDE to increase the levels of amyloid beta in the brain [*28*]. Craft *et al.* reported that insulin stimulates inflammation in the CNS [*29*].

Involvement of cytokines

We performed a study to elucidate the mechanism of DM-related cognitive decline [*30*]. Inflammatory mechanisms have been hypothesized to play a role in the pathogenesis of several age-associated diseases, including Alzheimer's disease [*31*]. Additionally, high plasma levels of inflammatory proteins reportedly increase the risk of cognitive decline in both non-DM [*32, 33*] and DM subjects [*34*], although the involvement of inflammatory cytokines in DM-related cognitive impairment has not yet been investigated.

We recruited 45 outpatients (25 males and 20 females) ranging in age from 65 to 85 years old (mean age, 72.6 ± 5.7). The average HbA1c and BMI were 7.0% and 24.6, respectively. The same exclusion criteria and cognitive assessment as those in Mogi *et al.* [*5*] were applied.

The clinical variables assessed were age, sex, years of education, DM duration, HbA1c, fasting serum glucose, immunoreactive insulin, body mass index, total cholesterol (TC), high-density lipoprotein cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, statin use, antihypertensive medication and smoking, and the existence of diabetic microangiopathic complications (neuropathy, nephropathy, retinopathy). The levels of serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were determined by commercially available enzyme-linked immunosorbent assays (Quantikine HS TNFα and Quantikine HS IL-6, R & D Systems, Minneapolis, MN, USA). High-sensitivity C-reactive protein (hs-CRP) was measured by latex-enhanced assay. Comparisons between two groups were made using the Student's t-test and χ-square analysis. Logistic regression analysis was performed to determine if the significant variables identified by the Student's t-test and the χ-square analysis were significant factors which would predict that the scores of the cognitive tests would be in the lowest quartiles.

The lowest quartile of WAIS-R scores was compared with the other three quartiles for every index (Table 4), and the group of subjects in the lowest quartile was found to have significantly higher serum levels of TC and TNF-α. Multivariate analysis using a model including significantly different factors from previous analyses showed that TNF-α was a significant and independent predictor of lower scores on the WASI-R symbol (Table 5).

The highest quartile of serum TNF-α levels showed significantly lower scores on the WAIS-R symbol test and MMSE (31.9 ± 9.1 vs. 38.9 ± 9.0 and 26.5 ± 1.1 vs. 27.9 ± 1.4, respectively).

Several studies on non-DM subjects have demonstrated that inflammatory markers are associated with cognitive impairment [*35, 36*], and a recent study on elderly people with metabolic syndrome revealed that high inflammation was a significant factor associated with cognitive impairment [*37*]. Because the overexpression of IL-6 showed progressive neuronal loss and decreased learning in an animal model [*38*], is possible that inflammation itself could affect cognitive ability.

Another potential mechanism could be through atherosclerosis. Inflammatory markers, including TNF-α, have been suggested to be involved in the process of atherogenesis [*39, 40*], and a recent trial showed that treatment with TNF-α blockers decreased cardiovascular events [*41*]. Serum inflammatory markers, including TNF-α, were found to be high in patients with brain infarction [*42*], and one study has demonstrated a relationship between inflammatory proteins and silent brain infarctions [*43*]. In the present study, all potential subjects with a clinical history of strokes and/or focal neurological signs were excluded, but subjects with high serum TNF-α might have silent brain infarctions, which could affect cognitive functions.

A recent population-based study reported that moderate-to-high physical activity was associated with low inflammatory markers [*44*]. Interestingly, physical activity is reportedly a protective factor in cognitive decline and dementia [*45, 46*].

Conclusion

The accumulating evidence indicates that DM is associated with cognitive decline, and may be a risk factor for dementia of the Alzheimer's type and vascular origin. We do not yet know, however, how to intervene to prevent DM-related cognitive decline or dementia, or how to reverse it once it occurs. The mechanism of brain dysfunction asso-

Table 4.

	Higher	Lowest	P value
n	34	11	
Age (yrs)	72.1 ± 5.3	74.7 ± 7.3	0.19
Education (yrs)	10.2 ± 2.2	8.7 ± 1.8	0.53
DM duration	16.9 ± 7.3	17.9 ± 6.2	0.68
HbA1c (%)	7.2 ± 1.4	6.6 ± 1.2	0.20
FBS (mg/dl)	144 ± 38.0	138.2 ± 50.6	0.68
IRI	10.9 ± 6.3	8.3 ± 5.5	0.22
BMI (kg/m^2)	24.4 ± 3.4	25.3 ± 3.9	0.45
TC (mg/dl)	193.1 ± 41.7	221.9 ± 29.9	0.04
HDL (mg/dl)	48.7 ± 12.0	53.3 ± 16.4	0.31
TG (mg/dl)	148.9 ± 84.2	215 ± 299.5	0.24
SBP (mmHg)	132.2 ± 14.8	134.0 ± 13.0	0.72
DBP (mmHg)	74.4 ± 8.4	70.3 ± 10.2	0.18
TNF-α (Log)	−0.09 ± 0.31	0.18 ± 0.31	0.01
IL-6 (Log)	0.35 ± 0.21	0.46 ± 0.15	0.11
H CRP (Log)	−1.03 ± 0.51	−1.03 ± 0.37	0.99
Sex (male %)	19 (55.8)	7 (63.6)	0.84
Neuropathy (%)	20 (58.8)	7 (63.4)	0.98
Retinopathy (%)	20 (58.8)	8 (72.7)	0.57
Nephropathy (%)	9 (26.5)	5 (45.5)	0.39
Antihypertensive medication (%)	18 (52.9)	9 (81.8)	0.15
Statin use (%)	13 (38.2)	3 (27.3)	0.81
Smoker (%)	12 (35.3)	6 (54.5)	0.40

Data are the means ± SD

Table 5. Odds ratio

	Exp (95% CI)	P value
TNF-α	44.49 (1.38–1426.30)	0.03
TC	1.02 (1.00–1.05)	0.06

ciated with DM needs to be investigated fully.

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Association between insulin resistance and cognitive function in elderly diabetic patients

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Background: Recently, cognitive impairment in elder diabetic subjects has sparked considerable interest. Insulin resistance (IR) is one of the central pathologies in diabetes mellitus, and several studies have shown that IR is associated with cognitive impairment in non-diabetic elderly subjects. However, the involvement of IR in cognitive dysfunction in the diabetic elderly has remained to be elucidated.

Methods: In the current study we measured IR with the euglycemic insulin clamp technique, and assessed cognitive function in 13 elderly diabetic patients (mean age, 69.1 ± 4.4). Several tests to assess cognitive function including Mini-Mental State Examination (MMSE) were performed, and clinical indices were evaluated. IR was evaluated by metabolic clearance rates (MCR).

Results: The Spearman's rank correlation coefficient between MCR and MMSE scores was 0.587 ($P = 0.035$). When subjects were divided into two groups at the median MCR (5.0 mL/kg/min), the lower MCR (high IR) group ($n = 5$) had significantly lower MMSE scores than the higher group ($n = 8$). The Spearman's rank correlation coefficient was −0.641 ($P = 0.018$) between hs-CRP and MMSE scores. When subjects were divided into two groups at the median of high-sensitivity C-reactive protein (hs-CRP) (594.0 μg/dL), the higher hs-CRP group ($n = 6$) had significantly lower MMSE scores than the lower group ($n = 7$).

Conclusion: The current study shows that higher IR measured with the euglycemic insulin clamp technique and higher hs-CRP is associated with lower MMSE scores in non-demented diabetic elderly patients.

Keywords: C-reactive protein, type 2 diabetes mellitus, euglycemic clamp technique, insulin resistance, neuropsychological test.

Introduction

The number of elderly diabetic patients has been increasing in most developed countries including Japan. In diabetic pathology there are several kinds of abnormalities of the metabolism, mainly the glucose metabolism; these induce systemic complications, namely microangiopathy and macroangiopathy. At the center of the pathophysiology of type 2 diabetes is insulin resistance and compensatory hyperinsulinemia; these pathologies accelerate atherosclerosis through abnormal vascular relaxation, increase of oxidative stress, activation of the sympathetic nervous system, and increased sodium reabsorption. Recently, cognitive impairment in elder diabetic subjects has sparked

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considerable interest. Several studies have shown that diabetes is a risk for cognitive decline and incidents of dementia, not only of vascular origin, but also of Alzheimer-type.[1,2]

The pathogenesis of diabetes-related cognitive impairment has not been fully elucidated; however, several hypotheses have been raised. The involvement of insulin resistance (IR) is one of these. Increasing evidence suggests that IR and/or elevated insulin levels may be associated with adverse effects on cognition in non-diabetic elderly subjects.[3–5]

Although IR is evaluated by several methods such as the homeostatic model assessment (HOMA-R), the euglycemic insulin clamp technique is the gold standard for IR measurement.[6] While some studies investigated the association between IR and cognition,[3–5] no studies have used the euglycemic insulin clamp technique to assess the relationship between IR and cognitive function in diabetic patients.

In the current study we measured IR with the euglycemic insulin clamp technique, and assessed cognitive function in non-demented elderly diabetic patients.

Methods

Subjects

Thirteen elderly diabetic outpatients (six males and seven females) whose ages ranged between 65 and 77 (mean age, 69.1 ± 4.4) were enrolled in the study. Subjects with a diagnosis of dementia according to the Diagnostic and Statistical Manual of Mental Disorders Third Edition Revised (DSM-III-R) for dementia[7] and/or whose score on the Mini Mental State Examination (MMSE) was 23 or lower were excluded, as were those who had a clinical history and/or neurological symptoms of stroke. Subjects with the diagnosis or treatment of depression were not involved. No subjects had difficulty in undergoing a cognitive functional assessment of audio-visual deficiency. The users of thiazolidine derivatives or metformin were excluded.

An ethical committee in the Department of Health Science, Faculty of Psychological and Physical Science, Aichi Gakuin University approved our study, and all patients gave their written informed consent prior to participation. Cognitive functional tests were administered individually to each subject. Assessment was performed in the morning, after the subjects had eaten their usual breakfast and had been examined by a doctor to ascertain their physical condition.

Cognitive tests

Cognitive function was assessed by structured performance tests that were selected to represent a broad range of cognitive domains, including those measured in previous studies of type 2 diabetes, as reviewed by Strachan *et al.*[8] The MMSE,[9] which assesses orientation, registration, attention, calculation, language and recall with a score range from 0 to 30, was used to assess mental status. For verbal memory, we used the Word List, a subtest of the Alzheimer's Disease Assessment Scale (ADAS).[10] Paragraph recall (immediate and delayed) from the neuropsychological tests of the National Center of Neurology and Psychiatry, Japan[11] was also assessed. Complex psychomotor skills were evaluated by the Digit Symbol Test, a subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R).[12] We used the Stroop Color-Word Test[13] (Japanese version) to assess attention. Seconds to completion are recorded and the difference between the time required to read the word card and that required to read the dots card is calculated.

A well-trained psychological tester administered all four tests in the same order for all subjects. The doctors checked the physical conditions of the subjects before the assessment and confirmed that they were not hypoglycemic.

Glucose infusion rates (GIR) and metabolic clearance rates (MCR) were determined by the euglycemic clamp procedure.

Insulin action was evaluated using the euglycemic clamp, a method that has already been described in detail.[14] Briefly, after an overnight fast, an intravenous catheter was inserted into an antecubital vein for infusion of insulin and glucose. Regular human insulin (Novolin-R, Novo Nordisk A/S, Denmark) was continuously infused using a continuous-injection pump at a rate of 40 mU/m^2/min. The blood glucose concentration was measured at 5-min intervals during the clamp procedure using a glucose monitor (G sensor, GLUCOCARD G meter, ARKLAY, Inc., Japan), and it was maintained at the fasting level by adjusting the infusion rate of a 20% glucose solution. Given that the hepatic glucose output has been shown to be suppressed more than 90% at 40 mU/m^2/min insulin infusion, the GIR could be regarded as a measure of the peripheral glucose uptake.[15] In order to compare *in vivo* insulin action of subjects with different basal plasma glucose levels, MCR were calculated by dividing GIR by the mean plasma glucose.[16] Generally, a lower MCR means a higher IR.

The euglycemic clamp and the cognitive assessment were performed on the same day.

Clinical characteristics

Covariants examined were age, sex, years of education, duration of diabetes, HbA1c, fasting blood sugar (FBS), immunoreactive insulin (IRI), body mass index (BMI), total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglyceride (TG), systolic blood pressure (SBP), diastolic blood pressure (DBP), statin use,

antihypertensive medication and smoking. IRI was measured only in non-insulin users. High-sensitivity C-reactive protein (hs-CRP) was measured by latex-enhanced assay.[17]

Statistical analysis

All data are presented as mean ± standard deviation (SD). All variables were divided into two groups at the median value, and compared by Mann–Whitney's U analysis. Spearman's rank correlation coefficient was calculated to confirm the relationship between the variables. A P-value of less than 0.05 was considered to indicate statistical significance.

Results

The characteristics of the patients are shown in Table 1. The mean HbA1c and BMI were 7.6 ± 0.6% and 24.9 ± 8.1, respectively. The mean score of MMSE was 26.9 ± 1.5, and the mean MCR was 4.9 ± 1.2 mL/kg/min.

MMSE scores and MCR were significantly correlated (Spearman's rank correlation coefficient = 0.587, $P = 0.035$) (Fig. 1). The subjects were divided into two groups at the median MCR (5.0 mL/kg/min), and all other variables were compared. MMSE scores were significantly lower in the group with lower MCR values (higher IR) (25.5 ± 0.6, $n = 8$) than in those with higher MCR (27.8 ± 1.0, $n = 5$).

The MMSE scores and hs-CRP were significantly inversely correlated (Spearman's rank correlation coefficient = −0.641, $P = 0.018$) (Fig. 2). The subjects were divided into two groups at the median hs-CRP (594.0 μg/dL), and all other variables were compared. MMSE scores were significantly lower in the group with higher hs-CRP (26.2 ± 1.2, $n = 7$) than in that with lower hs-CRP (28.2 ± 1.3, $n = 6$).

No significant association was found between MCR and hs-CRP ($P = 0.256$). Other cognitive test scores had no significant associations with MCR or hs-CRP. BMI did not have significant correlations with MCR or hs-CRP ($P = 0.115$, 0.291, respectively). Specific data are shown in Table 2.

Table 1 Clinical characteristics of the participants

Sex (male/female)	6/7
Age (years)	69.1 ± 4.4
Duration of diabetes (years)	16.9 ± 7.9
Therapy (diet/OHAs/insulin)	4/6/3
Body mass index (kg/m^2)	22.5 ± 8.2
Systolic blood pressure (mmHg)	128.0 ± 12.6
Diastolic blood pressure (mmHg)	78.1 ± 6.6
HbA1c (%)	7.6 ± 0.6
FBS (mg/dL)	132.6 ± 33.0
IRI (μU/mL)	11.7 ± 9.0
TC (mg/dL)	204.2 ± 25.8
HDL (mg/dL)	54.1 ± 14.6
TG (mg/dL)	126.4 ± 76.4
MCR (ml/kg/min)	4.9 ± 1.2
hs-CRP(μg/dL)	846.2 ± 219.2

Values are expressed as means ± SD.
BMI, body mass index; FBS, fasting blood sugar; HDL, high density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IRI, immunoreactive insulin; MCR, metabolic clearance rates; OHA, oral hypoglycemic agents; TC, total cholesterol; TG, triglyceride.

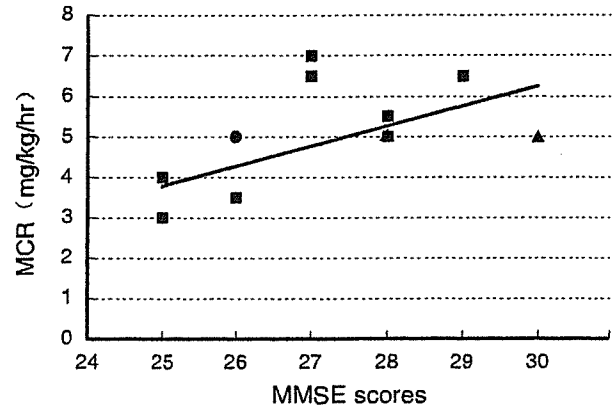

Figure 1 The association of Mini-Mental State Examination (MMSE) scores and metabolic clearance rates (MCR). The MMSE scores and MCR measurement were significantly correlated. Spearman's rank correlation coefficient was 0.587 ($P = 0.035$). ●, diet; ■, oral hypoglycemic agents (OHA); ▲, insulin.

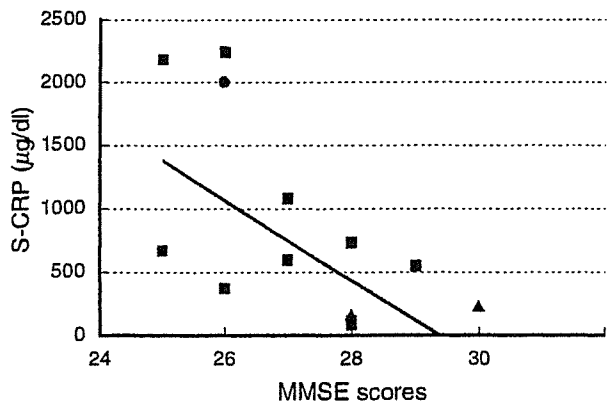

Figure 2 The association of Mini-Mental State Examination (MMSE) scores and high-sensitivity C-reactive protein (hs-CRP). The MMSE scores and metabolic clearance rates (MCR) measurement were significantly correlated. Spearman's rank correlation coefficient was −0.641 ($P = 0.018$). ●, diet; ■, oral hypoglycemic agents (OHA); ▲, insulin.

Table 2 Results of individual data

	MCR		hs-CRP	
	MCR < 5	MCR ≥ 5	S-CRP ≥ 594	S-CRP < 594
Sex (male/female)	3/1	3/6	2/4	4/3
Age (years)	69.1 ± 4.1	69.0 ± 5.7	66.9 ± 3.7	71.7 ± 3.8
Duration of diabetes (years)	16.3 ± 8.4	18.3 ± 7.7	16.4 ± 6.9	17.5 ± 9.6
HbA1c (%)	7.6 ± 0.7	7.5 ± 0.5	7.5 ± 0.8	7.7 ± 0.4
Body mass index (kg/m^2)	27.9 ± 9.0	23.2 ± 5.3	29.6 ± 9.0	22.8 ± 5.6
Systolic blood pressure (mmHg)	132.9 ± 10.59	117.0 ± 10.1	129.3 ± 14.7	126.5 ± 10.7
Diastolic blood pressure (mmHg)	80.0 ± 6.9	73.8 ± 3.8	78.4 ± 5.5	77.7 ± 8.3
TC (mg/dL)	208.2 ± 28.8	195.3 ± 17.5	212.3 ± 29.0	194.8 ± 19.8
HDL (mg/dL)	56.2 ± 14.6	49.3 ± 15.435	49.0 ± 13.5	60.0 ± 14.6
TG (mg/dL)	118.9 ± 58.2	143.3 ± 117.4	160.7 ± 62.4	86.3 ± 75.9
IRI (μU/mL)	13.6 ± 10.1	7.5 ± 4.3	11.4 ± 9.5	12.1 ± 9.4
FBS (mg/dL)	127.6 ± 33.1	144.0 ± 34.4	131.9 ± 30.2	133.5 ± 38.9
MCR (ml/kg/min)	4.3 ± 0.8	6.4 ± 0.6	4.9 ± 1.5	5.1 ± 1.0
hs-CRP(μg/dL)	966.2 ± 906.6	576.3 ± 407.9	1355.9 ± 752.6	251.7 ± 177.8
MMSE	26.9 ± 1.7	27.8 ± 1.0	26.3 ± 1.1	28.2 ± 1.3
ADAS (immediate)	7.1 ± 1.1	6.3 ± 1.0	7.0 ± 1.1	6.6 ± 1.2
ADAS (delayed)	6.8 ± 2.0	7.3 ± 1.9	7.1 ± 2.0	6.7 ± 1.9
WAIS-R Symbol	48.1 ± 9.8	40.5 ± 13.2	41.6 ± 12.3	50.7 ± 7.4
Paragraph recall (immediate)	8.3 ± 3.0	5.6 ± 1.8	7.4 ± 3.5	7.6 ± 2.3
Paragraph recall (delayed)	6.6 ± 3.7	4.4 ± 1.4	6.0 ± 3.8	5.8 ± 3.0
Stroop test (seconds)	12.9 ± 6.5	13.1 ± 7.0	14.1 ± 7.1	11.6 ± 5.7

Values are expressed as means ± SD.
ADAS, Alzheimer's Disease Assessment Scale; BMI, body mass index; FBS, fasting blood sugar; HDL, high density lipoprotein cholesterol; hs-CRP, high sensitivity c reactive protein; IRI, immunoreactive insulin; MMSE, Mini-Metal State Examination; TC, total cholesterol; TG, triglyceride.

Discussion

In the present study MMSE scores in non-demented elderly diabetic subjects were significantly correlated with MCR and hs-CRP. The group with lower MCR had significantly lower MMSE scores, and the higher hs-CRP group had significantly lower MMSE scores. These results suggested that in the non-demented elderly diabetic subjects who are at high risk for cognitive impairment, IR and/or inflammation were associated with cognitive function.

Previous studies have reported an association between IR and cognitive impairment in non-diabetic subjects based on plasma insulin measurement, HOMA-R, or the Quantitative Insulin Sensitivity Check Index.[18] The current study assessed IR using the euglycemia clamp technique, which is a gold standard for IR assessment, and demonstrated significant correlation between IR and MMSE scores in non-demented elderly diabetic patients.

The mechanism of the involvement of IR in cognitive impairment remains to be elucidated; however, several hypotheses have been raised. First, IR accelerates atherosclerosis,[19] and asymptomatic cerebrovascular lesions may lead to cognitive decline. Second, IR-induced compensatory hyperinsulinemia may be involved. Amyloid beta is a neurotoxic substrate that plays a critical role in the pathogenesis of dementia of Alzheimer type. Insulin degrading enzyme (IDE) reportedly degrades in the central nervous system, and an excess amount of insulin may lead to a decrease of amyloid beta degeneration by IDE with a competitive mechanism.[20] The current study showed that IR was associated with cognitive decline in elderly diabetic patients; however, the mechanism still remains unknown, and further study is required to investigate how IR is associated with cognitive decline in non-diabetic and diabetic elderly persons.

Several studies have reported that inflammation is associated with cognitive impairment in non-diabetic subjects,[21,22] and recently in elderly subjects with metabolic syndrome,[23] a higher inflammatory marker was shown to be a risk factor for cognitive decline. The links between systemic inflammation and cognitive decline also remain largely unknown. Inflammation is associated with atherosclerosis,[24] again, inflammation may be associated with cognitive impairment through a vascular mechanism. Indeed, one study has demonstrated a relationship between inflammatory proteins and silent brain infarctions.[25] Because overexpression of IL-6, which is

an inflammatory cytokine, showed progressive neuronal loss and decreased learning in an animal model,[26] it is possible that inflammation itself could affect cognitive ability. One study demonstrated that hyperinsulinemia provokes an increase of inflammatory cytokines and amyloid beta in the periphery and the brain.[27]

In the current study we did not find a statistically significant relationship between IR (MCR) and inflammation (hs-CRP). Many previous reports have indicated a definite relation between IR and inflammation;[28] therefore, further investigation is required.

The reasons why only MMSE scores but not other types of cognitive functional assessment were associated with IR or inflammation were not clear; however, this may suggest that factors other than IR or inflammation contribute to diabetes-related cognitive dysfunction.

The current study had several limitations. First, because of the cross-sectional study design, it was unclear whether treatment on IR and/or inflammation leads to cognitive functional recovery. Many studies have demonstrated that exercise improves IR in the elderly.[29,30] Interestingly, several observational studies reported that higher physical activities were associated with reduced risk for dementia.[31,32] It is important to investigate whether improving IR by exercise can ameliorate cognitive impairment. Second, because the number of patients involved was relatively small, the influence of antidiabetic medication and/or other possible confounding factors could not be assessed. In the current study the users of thiazolidine derivatives or metformin were excluded, however, other drugs including statins or the inhibitors of the angiotensin system could affect IR or inflammation.[33] Therefore, the effects of these drugs and other possible confounding factors will be studied with more subjects in the future. The Rotterdam study reported that patients with insulin treatment had a higher risk of dementia.[34] The effects of exogenous insulin on cognitive function should also be further investigated. Third, brain imaging was not available in the current study although the subjects with a history of stroke were excluded. Asymptomatic brain infarctions may be involved in IR-mediated cognitive dysfunction, and future studies will be needed. Finally, because the subjects with very mild Alzheimer's disease and other degenerative diseases could not be excluded in the current study, further follow-up study may be needed.

In summary, higher IR measured with the euglycemic insulin clamp technique and higher hs-CRP were both associated with lower MMSE scores in the diabetic elderly.

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