DM-related cognitive decline, the factors associated with this condition must first be determined. In the present study, we investigated factors associated with cognitive impairment in elderly DM subjects using baseline data from a large-scale cohort study of elderly DM in Japan.

2. Methods

2.1. Participants

The J-EDIT study was initiated in 2001 as a prospective intervention study of elderly Japanese people with DM for the purpose of determining how to prevent several diabetic complications. One thousand one hundred and seventy-three diabetic subjects were enrolled in 39 institutes and hospitals in Japan. They were all aged 65 years or more and had serum HbA1c levels at least 7.5%, or at least 7.0% with one of the following comorbidity factors: hypertension (130/85 mmHg and over), obesity (a body mass index (BMI) of at least 25), dyslipidemia (total cholesterol of at least 200 mg/dl, low-density lipoprotein (LDL) of at least 120 mg/dl, high-density lipoprotein (HDL) of 40 mg/dl or less, and/or triglyceride of at least 150 mg/dl). Although no exclusion criteria were determined for the registration of JEDIT, severely demented subjects were not selected because the filling out of several questionnaires was mandatory.

The study protocol was approved by the ethical committee in all of the enrolled institutes, and written informed consent was obtained from each patient.

2.2. Functional assessment

The Mini Mental State Examination (MMSE) was administered to most patients (907 of 1173) upon registration (Folstein et al., 1978). The MMSE is a global test of orientation, attention, calculation, language and recall with a score of 0–30.

Of the 1173 enrolled cases, MMSE scores were not collected in 266; data sheets were not returned in 48, subjects dropped out just after registration in 35, and doctors did not perform MMSE in 183.

Basic activities of daily living (BADL) was measured by a Barthel Index score of 0–20 (Mahoney and Barthel, 1965), and depressive mood was assessed by a short version of the Geriatric Depression Scale (GDS-15) (Yesavage, 1986).

2.3. Assessment of diabetes mellitus, complications and comorbidities

The diagnosis and patient data regarding DM, blood examinations and complications were obtained from clinical charts (The Expert Committee, 2003). After overnight fasting, blood samples were taken by venipuncture to assess serum levels of glucose, HbA1c, total cholesterol, triglyceride and HDL cholesterol. Additionally, serum insulin concentrations were determined in patients who were not receiving insulin therapy. Diabetic nephropathy was assessed according to the mean urinary albumin-to-creatinine ratio (ACR) and was classified as no nephropathy (ACR < 30 μg/mg) or existence of nephropathy (microalbuminemia: 30 ACR < 300 μg/mg or more advanced). Diabetic retinopathy was assessed by fundoscopic examination performed through dilated pupils by experienced ophthalmologists, and was classified into two categories: mild (no retinopathy or intraretinal hemorrhages and hard exudates), or serious (soft exudates, intraretinal microvascular abnormalities, venous calibre abnormalities, venous beading, neovascularization of the disc or other areas in the retina, preretinal fibrous tissue proliferation, preretinal or vitreous hemorrhage, and/or retinal detachment). Diabetic neuropathy was defined as either the loss of the Achilles tendon reflex without neuropathic symptoms including paresthesia, or the presence of neuropathic symptoms. Macrovascular complications were classified based on the presence or absence of coronary artery diseases, and/or a history of stroke. The existence of a current regular occupation and current habits of smoking, drinking and exercising were also assessed by questionnaire as yes (1) or no (0).

2.4. Statistical analysis

The subjects were divided into two groups, one with higher cognitive function, defined as having an MMSE score of 24 or more, and one with lower cognitive function, defined as having an MMSE score of 23 or less, according to the review by Tombaugh and McIntyre (1992). The groups were compared with respect to each factor by the Student's *t*-test for continuous variables or a χ^2-test for categorical variables. Logistic regression analysis including each factor as an explanatory variable was performed to search the association of the covariants and cognitive dysfunction indicated by an MMSE score below 24 after adjusting for age. Then, multiple logistic regression analysis was performed with the variables selected by this analysis and additional variables of interest. Spearman's rank correlation coefficient was calculated to confirm the relationship between serum albumin levels and MMSE scores.

3. Results

The background characteristics of the two MMSE score groups are shown in Tables 1 and 2. The average age was 74.0 years old in the lower MMSE-score group (23 and less) and 71.8 years old in the higher MMSE-score group (24 and more) (Table 1). The average HbA1c and FBG levels in the higher MMSE score group and lower MMSE score group were 8.0% versus 8.1% and 5.1 mmol/l versus 5.0 mmol/l, respectively (Table 1). At least about half of the participants had microangiopathic complications (nephropathy, retinopathy, or neuropathy) as shown in Table 2.

Table 1
Analysis by Student's *t*-test

Item	Higher	Lower	*p*-Value
Number	848	59	
Age (years)	71.8 ± 4.6	74.0 ± 5.1	<0.001
DM duration (years)	16.3 ± 9.7	17.1 ± 8.8	0.545
Height (cm)	155.8 ± 8.4	152.3 ± 8.6	0.002
Body weight (kg)	57.9 ± 10.2	57.7 ± 8.8	0.071
BMI	23.8 ± 3.5	23.9 ± 3.2	0.874
HbA1c (%)	8.0 ± 0.9	8.1 ± 1.1	0.766
FBG (mmol/l)	5.1 ± 0.3	5.0 ± 0.5	0.234
Systolic BP (mmHg)	135.4 ± 15.6	133.3 ± 19.3	0.391
Diastolic BP (mmHg)	74.9 ± 9.5	76.4 ± 11.2	0.288
LDL cholesterol (mg/dl)	120.9 ± 30.6	126.2 ± 35.7	0.201
HDL cholesterol (mg/dl)	56.4 ± 18.0	57.7 ± 18.4	0.567
Triglyceride (log)	4.7 ± 0.5	4.6 ± 0.5	0.353
Lp (a) (mg/dl)	23.1 ± 22.9	25.9 ± 23.5	0.362
Albumin (g/dl)	4.2 ± 0.4	4.1 ± 0.5	0.001
MMSE	28.5 ± 1.8	20.3 ± 3.0	<0.001
ADL	19.9 ± 3.4	18.9 ± 1.0	<0.001
GDS-15	4.0 ± 3.1	5.9 ± 3.9	<0.001

Higher: the group with higher MMSE scores (24 or more), Lower: the group with lower MMSE scores (23 or less).

Analysis by Student's *t*-test showed that age, height, activities of daily living (ADL) scores, and serum albumin were significantly different between the two groups of patients (Table 1). A history of cerebrovascular disease, existence of diabetic nephropathy, current smoking habit, current drinking habit, and absence of occupation were also demonstrated to have a significantly different distribution between the two groups (Table 2). Fasting serum insulin levels or insulin treatment were not significantly associated with MMSE scores.

To determine variables significantly associated with cognitive dysfunction, logistic regression analysis adjusted for age was performed. The variables selected by this analysis were age, body height, serum albumin, the existence of an occupation, smoking habits, drinking habits, the existence of nephropathy, GDS-15 scores and history of cerebrovascular disease (Table 3). Then, multiple regression analysis was performed with all these significant variables plus variables of interest (HbA1c and gender) considered simultaneously. As shown in Table 4, higher age, higher GDS-15 scores, lower serum albumin and a history of cerebrovascular disease were significantly associated with the group having lower MMSE scores.

Table 2
Analysis by χ^2-test

	Higher	Lower	*p*-Value
Male	45.9 (389)	40.0 (23)	0.304
Existence of current occupation	67.2 (552)	47.4 (27)	0.002
Existence of exercise habit	61.1 (497)	48.3 (28)	0.055
Current drinking habit	40.4 (343)	25.4 (15)	0.017
Current smoking habit	46.5 (383)	31.0 (18)	0.022
Existence of nephropathy	48.5 (411)	64.4 (38)	0.018
Existence of retinopathy	48.8 (413)	60.8 (35)	0.088
Existence of neuropathy	65.5 (544)	73.2 (41)	0.241
User of antihypertensive drugs	55.2 (468)	62.7 (37)	0.261
User of antidyslipidemic drugs	38.8 (329)	42.4 (25)	0.586
Antiplatlet user	26.9 (227)	49.2 (29)	<0.001
Presence of IHD	17.6 (149)	16.3 (9)	0.650
History of cerebrovascular disease	12.6 (107)	32.2 (19)	<0.001

Higher: the group with higher MMSE scores (24 or more). Lower: the group with lower MMSE scores (23 or less). Data are expressed as percentages of the total with the number in parentheses.

Table 3
Univariate regression analysis adjusted with age

	Odds ratio	95% CI	*p*-Value
Height (cm)	0.959	0.928–0.992	0.015
Gender (male)	1.232	0.714–2.126	0.453
HbA1c (%)	1.033	0.779–1.369	0.822
Systolic blood pressure (mmHg)	1.005	0.988–1.021	0.576
Diastolic blood pressure (mmHg)	1.017	0.990–1.044	0.230
Albumin (g/dl)	0.322	0.163–0.637	0.001
History of cerebrovascular disease	3.128	1.735–5.637	<0.001
Existence of nephropathy	1.877	1.079–3.264	0.026
Existence of retinopathy	1.730	0.998–2.997	0.051
Existence of neuropathy	1.369	0.742–2.527	0.315
Existence of current occupation	0.498	0.287–0.863	0.013
Current drinking habit	0.527	0.287–0.968	0.039
Current smoking habit	0.544	0.305–0.968	0.038
GDS-15	1.166	1.080–1.259	<0.001
ADL	1.019	0.998–1.042	0.0810

95% CI: 95% confidence interval.

Table 4
Multiple logistic regression analysis

	Odds ratio	95% CI	*p*-Value
Age (years)	1.079	1.011–1.150	0.021
Height (cm)	0.954	0.905–1.006	0.083
Gender (male)	0.429	0.139–1.323	0.141
Albumin (g/dl)	0.336	0.174–0.745	0.006
HbA1c (%)	0.965	0.703–1.325	0.828
History of cerebrovascular disease	3.011	1.578–5.748	<0.001
Existence of nephropathy	1.679	0.913–3.089	0.096
Existence of current occupation	0.725	0.348–1.368	0.321
Current smoking habit	0.516	0.223–1.195	0.123
Current drinking habit	0.601	0.274–1.315	0.202
GDS-15 scores	1.139	1.045–1.243	0.003

95% CI: 95% confidence interval.

MMSE scores and serum albumin levels were significantly correlated based on Spearman's correlation (coefficient = 0.14902, $p < 0.001$).

4. Discussion

The analysis of the data from the J-EDIT study at registration demonstrated that a history of cerebrovascular disease, a low serum albumin level, higher GDS scores, and higher age were independently associated with lower cognitive function.

The present study demonstrated that in DM subjects, the strongest risk factor for cognitive dysfunction as defined by a MMSE score less than 24, which is considered to be the level defining dementia (Tombaugh and McIntyre, 1992), was a

history of stroke. Although the causes of cognitive dysfunction were not determined in the present study, vascular lesions might play a prominent role in the cognitive decline of DM subjects with a history of stroke. Furthermore, Snowdon et al. report that among subjects who met the neuropathological criteria for Alzheimer's disease, those with brain infarcts had poorer cognitive functions and a higher prevalence of dementia (Snowdon et al., 1997); thus, cerebrovascular disease might shorten the period of preclinical dementia. In the current study the participants were all Japanese, a race which is relatively prone to cerebrovascular diseases (Kitamura et al., 2006). The prevalence of a history of stroke in the current study was 13.9% (126 out of 907 participants), much higher than the 1.8% reported by Kuusisto et al. (1994) in Finland, and comparable to 18.8% in PROACTIVE, a secondary prevention study for macrovascular disease in diabetic patients performed in European countries (Charbonnel et al., 2004). Thus, the higher stroke prevalence might have affected the results of the current study.

Lower levels of serum albumin, even within the "normal" range, are associated with increased risks of stroke and coronary heart disease incidents as well as all-cause and cardiovascular mortality (Shaper et al., 2004). Of particular interest are several lines of evidence demonstrating that chronic inflammation is involved in atherosclerotic mechanisms, and high-serum proinflammatory factors including c-reactive protein, interleukin-6 and tumor necrosis factor have been reported to be risk factors for progressed atherosclerosis; these proinflammatory factors reportedly suppress the synthesis of albumin in the liver (Chojkier, 2005). The present results indicate that lower serum albumin and a history of cerebrovascular disease are independent factors associated with cognitive decline. However, asymptomatic strokes may also be involved in the mechanism of cognitive impairment in elderly diabetic patients (Araki and Ito, 2002). Although lower serum albumin was strongly associated with cognitive decline, mean urinary ACR was not associated with MMSE scores (data not shown).

The scores of GDS-15, which assessed depressive mood, were significantly associated with lower MMSE scores. The association of a depressive mood with cognitive dysfunction has been reported (Jorm, 2000). However, the mechanism of this association remains to be elucidated (Jorm, 2000). Cognitive dysfunction and depression may share common risk factors, depression may be a risk factor or prodrome of cognitive dysfunction, depression may affect the threshold of cognitive dysfunction, or depression may be a causal factor in cognitive dysfunction. Further analysis of longitudinal data of JEDIT study may shed light on this subject.

Many population-based and clinical studies have shown that DM is associated with cognitive decline in the elderly (Cukierman et al., 2005; Mogi et al., 2004; Strachen et al., 1997). Several hypothetical mechanisms have been suggested for this impairment; however, their clinical relevance is unclear (Biessels et al., 2006). The J-EDIT study was an interventional prospective study with a randomized control design. Longitudinal clinical and cognitive assessment of elderly diabetic patients will provide more information on the mechanisms of DM-related cognitive disorders.

Some limitations should be considered in the present case. First, the present study was performed with cross-sectional design using the data obtained at registration for the J-EDIT study. The patients are being followed longitudinally, and a follow-up analysis will be reported in the future. Second, because all of the patients enrolled were diabetic, it was not clear whether or not the results of the present study were diabetes-specific. In particular, the involvement of low serum albumin in the mechanism of cognitive decline in non-diabetic elderly patients should be investigated. Third, the present study did not include brain imaging. A subgroup analysis of J-EDIT subjects who underwent brain magnetic resonance imaging (MRI) was recently reported elsewhere (Akisaki et al., 2006), and revealed that cognitive decline in diabetes was associated with white-matter hyperintensities and subcortical atrophy in the tested subgroup. However, the relationship between the present results and the results of MRI analysis requires further investigation.

In the present study, neither DM-specific clinical indices including HbA1c, fasting blood glucose and serum insulin level, nor DM-related microangiopathies (nephropathy, neuropathy, and retinopathy) were associated with lower MMSE scores. The J-EDIT study recruited patients with relatively severe DM status, and this group of patients therefore did not represent the general population of elderly diabetics. The criteria for diagnosis for microangiopathy in the present study were relatively simple. Retinopathy has been reported as being associated with cognitive impairment or brain atrophy (Musen et al., 2006; Wong et al., 2002); proteinuria, which is a symptom of diabetic nephropathy, has received attention as a risk for stroke and ischemic heart disease (Madison et al., 2006); dysfunctions of the central and peripheral nervous systems may share a common pathogenesis (Gispen and Biessel, 2000; Suzuki et al., 2006). Further investigation of subjects with a broader clinical background and more sensitive diagnostic criteria for DM-related microangiopathic complications is required.

In conclusion, based on the results obtained in the current cross-sectional assessment, the prevention of cerebrovascular disease may be a primary way of preventing cognitive decline in elderly DM subjects. An investigation of how lower serum albumin levels are associated with DM-related cognitive impairment may lead to the development of effective strategies for the prevention or treatment of this decline.

Conflict of interest declaration

There is no conflict of interest for any of the authors.

Acknowledgement

This work was supported by a Grant-in-Aid for Longevity Scientific Research H17-Cyouju-013 from the Ministry of Health, Labor and Welfare of Japan.

References

Areosa Sastre A., Grimley Evans J., 2007. Effects of treatment of type II diabetes mellitus on the development of cognitive impairment and dementia. The Cochrane Library, issue 2, http://www.cochrane.org/reviews/en/ab003804.html.

Akisaki, T., Sakurai, T., Takata, T., Umegaki, H., Araki, A., Mizuno, S., Tanaka, S., Ohashi, Y., Iguchi, A., Yokono, K., Ito, H., 2006. Cognitive dysfunction associates with white matter hyperintensities and subcortical atrophy on magnetic resonance imaging of the elderly diabetes mellitus Japanese Elderly Diabetes Intervention Trial (J-EDIT). Diabetes/Metab. Res. Rev. 22 (5), 376–384.

Araki, A., Ito, H., 2002. Asymptomatic cerebral infarction on brain MR images and cognitive function in elderly diabetic patients. Geriatr. Gerontol. Int. 2, 206–214.

Biessels, G.J., Staekenborg, S., Brunner, E., Brayne, C., Scheltens, P., 2006. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol. 5, 64–74.

Charbonnel, B., Dormandy, J., Erdmann, E., Massi-Benedetti, M., Skene, A., PROactive Study Group, 2004. The prospective pioglitazone clinical trial in macrovascular events (PROactive) can pioglitazone reduce cardiovascular events in diabetes? Study design and baseline characteristics of 5238 patients. Diabetes Care 27, 1647–1653.

Chojkier, M., 2005. Inhibition of albumin synthesis in chronic diseases: molecular mechanisms. J. Clin. Gastroenterol. 39 (4 suppl. 2), S143–S146.

Cukierman, T., Gerstein, H.C., Williamson, J.D., 2005. Cognitive decline and dementia in diabetes-systematic overview of prospective observational studies. Diabetologia 48, 2460–2469.

Folstein, M.F., Folstein, S.E., McHigh, P.R., 1978. 'Mini-Mental State': a practical method of grading the cognitive function of patients for the clinician. J. Psychiatr. Res. 12, 189–198.

Gispen, W.H., Biessel, G.J., 2000. Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci. 23, 542–549.

Jorm, A.F., 2000. Is depression a risk factor for dementia or cognitive decline? A review. Gerontology 46, 219–227.

Kitamura, A., Nakagawa, Y., Sato, M., Iso, H., Sato, S., Imano, H., Kiyama, M., Okada, T., Okada, H., Iida, M., Shimamoto, T., 2006. Proportions of stroke subtypes among men and women 40 years of age in an urban Japanese city in 1992, 1997, and 2002. Stroke 37, 1374–1378.

Kuusisto, J., Mykkanen, L., Pyorala, K., Laakso, M., 1994. Non-insulin-dependent diabetes and its metabolic control are important predictors of stroke in elderly subjects. Stroke 6, 1157–1164.

Madison, J.R., Spies, C., Schatz, I.J., Masaki, K., Chen, R., Yano, K., Curb, J.D., 2006. Proteinuria and risk for stroke and coronary heart disease during 27 years of follow-up: the Honolulu Heart Program. Arch. Intern. Med. 166, 884–889.

Mahoney, F.I., Barthel, D.W., 1965. Functional evaluation: the Barthel Index. Md. State Med. J. 14, 61–65.

Mogi, N., Umegaki, H., Hattori, A., Maeda, N., Miura, H., Kuzuya, M., Shimokata, H., Ando, F., Ito, H., Iguchi, A., 2004. Cognitive function in Japanese elderly with type 2 diabetes mellitus. J. Diabetes Complict. 18 (1), 42–46.

Musen, G., Lyoo, I.K., Sparks, C.R., Weinger, K., Hwang, J., Ryan, C.M., Jimerson, D.C., Hennen, J., Renshaw, P.F., Jacobson, A.M., 2006. Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. Diabetes 55, 326–333.

Shaper, A.G., Wannamethee, S.G., Whincup, P.H., 2004. Serum albumin and risk of stroke, coronary heart disease, and mortality: the role of cigarette smoking. J. Clin. Epidemiol. 57, 195–202.

Snowdon, D.A., Greiner, L.H., Mortimer, J.A., Riley, K.P., Greiner, P.A., Markesbery, W.R., 1997. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. JAMA 277 (10), 813–817.

Strachen, M.W., Ewing, F.M., Deary, I.J., et al., 1997. Is type 2 diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. Diabetes Care 20, 438–445.

Suzuki, M., Umegaki, H., Ieda, S., Mogi, N., Iguchi, A., 2006. Factors associated with cognitive impairment in elderly diabetes mellitus patients. J. Am. Geriatr. Soc. 54, 558–559.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 26, S5–S20.

Tombaugh, T.N., McIntyre, N.J., 1992. The mini-mental state examination: a comprehensive review. J. Am. Geriatr. Soc. 40, 922–935.

Wong, T.Y., Klein, R., Sharrett, A.R., Nieto, F.J., Boland, L.L., Couper, D.J., Mosley, T.H., Klein, B.E., Hubbard, L.D., Szklo, M., 2002. Retinal microvascular abnormalities and cognitive impairment in middle-aged persons: the atherosclerosis risk in communities study. Stroke 33, 1487–1492.

Yesavage, J.A., 1986. The use of self-rating depression scales in the elderly. In: Poon, L.W. (Ed.), Clinical Memory Assessment of Older Adults. American Psychological Association, Washington, DC, pp. 213–217.

Kobe J. Med. Sci., Vol. 54, No. 2, pp. E97-E107, 2008

The Double-edged Effect of Insulin on the Neuronal Cell Death Associated with Hypoglycemia on the Hippocampal Slice Culture

YOICHIRO TANAKA, TOSHIHIRO TAKATA, TOYOKA SATOMI, TAKASHI SAKURAI, and KOICHI YOKONO

Department of Internal and Geriatric Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Received 27 December 2007 /Accepted 9 January 2008

Key Words: Insulin, CA1, DG, hypoglycemia, neuronal cell death

It is well known that the central nervous system (CNS) is vulnerable to hypoglycemia and hyperglycemia. Insulin is indispensable for serum glucose control and diabetes patients are on the relative or absolute deficient state of insulin. The role of insulin on the CNS, however, has not been fully elucidated, yet. To reveal the role of insulin on the neuronal survival, we have used in vitro system of an organotypic hippocampal slice culture from rat, and examine the neuronal cell death at the various glucose concentrations in the presence or absence of insulin. When glucose concentrations is varied to 0, 1, 3, 5 and 30mM in the incubation medium, the neuronal cell death was minimum at 5mM, and no neuronal survival was observed under 1mM on the CA1. On the dentate gyrus granule cells (DG), on the other hand, the significant neuronal survival was observed even as low as 1mM. In the presence of 1nM concentration of insulin, the neuronal cell death curve showed the U-shape, and the minimum death point was 3-5mM glucose concentrations at the CA1. At the DG, insulin did not show the protective effect up to 48 hours culture regardless of glucose concentration. In the absence of glucose, insulin accelerated the neuronal cell death both in the CA1 and DG. We concluded that insulin has a double-edged effect on the neuronal cell death dependent on glucose concentration, and that the CA1 and the DG have a different sensitivity to insulin in terms of cell survival.

It has been well known that the central nervous system is vulnerable to hypoglycemia. Insulin regulates a blood glucose level and its deficiency causes diabetes. An action of insulin on the central nervous system has not been enough elucidated, yet. Recent reports have suggested that the type 2 diabetes is one of the risk factors for the decay of cognitive function and the blockade of insulin signal cascade may be involved for its pathology (17, 21, 22). And after ischemic events, insulin acts directly on the brain to reduce ischemic brain necrosis independent of hypoglycemia (26). Hypoglycemia causes lethal consequences during insulin treatment. Clinically, it is mandatory to avoid nocturnal hypoglycemia especially in case of treating elderly and the type1 diabetic patients, and it is known that 2 to 4% of the type1 diabetic patients die by an excess of insulin administration (15). The harm effects of hypoglycemia, therefore, are well known and the protection method is mainly to keep blood glucose in adequate levels. The insulin action per se during hypoglycemia against the CNS, however, is merely understood.

Phone: +81-78-382-5901 Fax: +81-78-382-5919 E-mail: h_m_0417@hotmail.com

In this paper, we examined the direct action of insulin on the neuronal cell death at a variety of glucose concentrations by using cultured hippocampal slices. Our experimental results suggested that the CNS damage during hypoglycemia would exaggerate by insulin therapy itself and the caution may be necessary not only the glucose levels but also the insulin therapy itself during hypoglycemia.

MATERIALS AND METHODS

The experiments were conducted according to the guidelines for animal experimentation at the Kobe University School of Medicine, and conform to the relevant National Institution of Health guidelines.

Preparation of organotypic hippocampal slices

Hippocampal slices were made from the septal half of the hippocampus using a standard method (20). Briefly, 9- to 11-days Wistar rats (Hartley, SLC, Japan) were anesthetized with 98% diethyl I ether and decapitated. The hippocampus were rapidly dissected at 4-6 °C and cut into 450μm slices using a McIllwain Tissue Chopper (Mickle Laboratory Engineering Co., Ltd, UK). Slices were then transferred onto membranes (pore diameter: 30μm, Millicell-CM, Millipore, Bedford, MA, USA), and placed into a six-well microplate (Costar Corning Inc, NY, USA) with 1mL of slice culture medium per well. The culture medium contained 50% Eagle's minimal essential medium (MEM) (Gibco, CA, USA), 25% Hanks' Balanced Salt Solution (HBSS)(Gibco, CA, USA), 25% heat-inactivated horse serum (Gibco, CA, USA) containing 1% penicillin/streptomycin. The medium was changed every 3 days. Slices were kept in culture for 14 days before study and the six-well microplates were stored at 37 °C in a 5% CO2 incubator under a 95% humidity atmosphere (Sanyo, Tokyo, Japan).

Treatment of hippocampal slices

Slices in the six-well microplates at day 14 were washed, and the basic medium was replaced with various agents for the treatment. The basic medium contained 90mM NaCl, 4mM KCl, 0.1mM MgCl2, 0.1mM KH2PO4, 0.5mM MgSO4, 0.1mM Na2HPO4, 0.5mM NaH2PO4, 14mM NaHCO3, 1.2mM CaCl2, 10mM glucose, about 2mM essential and non-essential amino acids, and 0.02mM vitamins. In order to investigate the changes in neuronal toxicity due to the glucose concentration, various glucose concentrations (0mM, 1mM, 3mM, 5mM, and 30mM) were added to the medium that was used to treat the slices. Moreover, the change in the neuronal death rate was investigated both with and without insulin loading at a concentration of 1nM. (insulin: Humulin® R, Eli Lilly, Indiana, USA)

Assessment of cell death in hippocampal slices

The propidium iodide (PI) method was used in the assessment of neuronal death in hippocampal slices at 24h, 48h, and 72h after each treatment in the CA1 and the dentate gyrus granule cells (DG) of the hippocampus. To label the nuclei of dead neurons, 4.6μg/mL of PI (Sigma, St. Louis, Mo, USA) was added to the wells of the culture microplates for 15min. PI is a polar compound that only enters cells with damaged cell membranes, where it binds to nucleic acids within the cells and develops a bright red fluorescence. The dye is basically non-toxic to neurons, and is used as an indicator of neuronal integrity and cell viability (11). Thus the intensity of fluorescence correlates with the cell death fraction. After 15 min, digital images of the PI fluorescence were obtained with an inverted fluorescence microscope (4×objective) equipped with a digital camera (Olympus IX70, Tokyo, Japan). After the final image, all the neurons were killed by adding 10 μ M N-methyl-D-aspartic acid (NMDA) and the final PI fluorescence intensity was

calibrated as 100% cell death. The mean intensity (green values) of the PI fluorescence were measured using an image program MacScope (Ver 2.6.1, Mitani Inc, Osaka, Japan).

Statistical analysis

Values were expressed as mean ± standard error of the mean (SEM) from three independent experiments. The statistical significance was established by ANOVA followed by a post-hoc test, and then the non-paired t-test was employed using StatView software (v.5.0.1.0; SAS Institute Inc., Cary, NC, USA). $p<0.05$ was considered to be statistically significant.

RESULTS

CA1 neuronal cell death in the presence or absence of serum

Serum is widely used for maintenance of cultured neuronal cell viability. To know the extent of nerve protection effects of serum in our experimental settings, the neuronal cell death was evaluated in the presence or absence of the heat inactivated horse serum (Gibco, CA, USA) in the culture medium. The glucose concentration in the medium was kept at 30mM, the concentration that is usually commercially available. After 72 hour culture, the neuronal cell survival was better in the presence of serum (n=30) in comparison with the absence of serum (n=11) and the cell death rate was 22.7 ± 6.3% and 40.8 ± 6.2%, respectively (non-paired t-test; $p<0.05$) (Fig.1) .

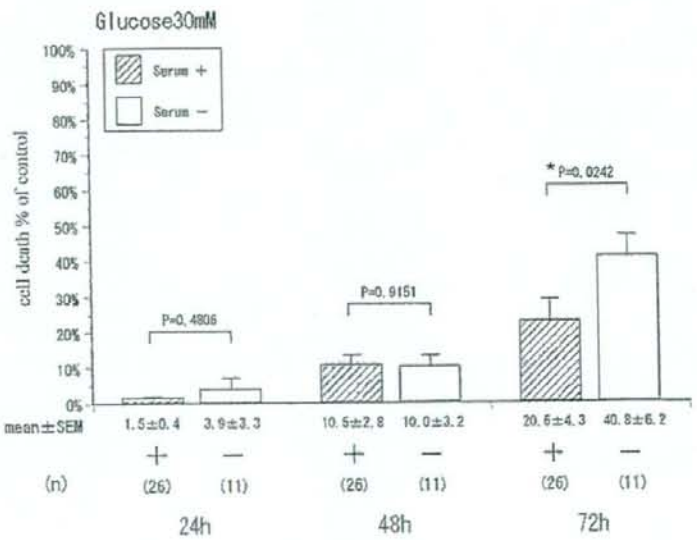

Fig. 1. CA1 neuronal cell death in the presence or absence of serum.
The glucose concentration in the medium was kept at 30mM, the concentration that is usually commercially available. After 72-hour culture, the neuronal cell survival was better in the presence of serum (n=30) in comparison with the absence of serum (n=11) and the cell death rate was 22.7±6.3% and 40.8±6.2%, respectively (*: non-paired t-test; $p<0.05$.)

The CA1 neuronal cell death during low glucose

The difference in cell death rates was examined in various glucose concentrations (0mM, 1mM, 3mM, 5mM, and 30mM) under the environment lacking serum on the CA1 pyramidal cell. Glucose 0mM (n=9) and 1mM (n=14) resulted prominently high cell death rates after 48 hour culture and the cell death rates were 57.0±6.5% and 53.7±7.4%, respectively. After 72 hour, the rate further increased and the each rate was 83.6± 4.9% and 92.9±1.2%, respectively. The cell death rates showed the U-shaped curve against the glucose concentrations and neuronal cell death was minimum at 5mM glucose (n=9) (Fig. 2)(One-way ANOVA; $p<0.0001$, Scheffe's F test; $p<0.01$).

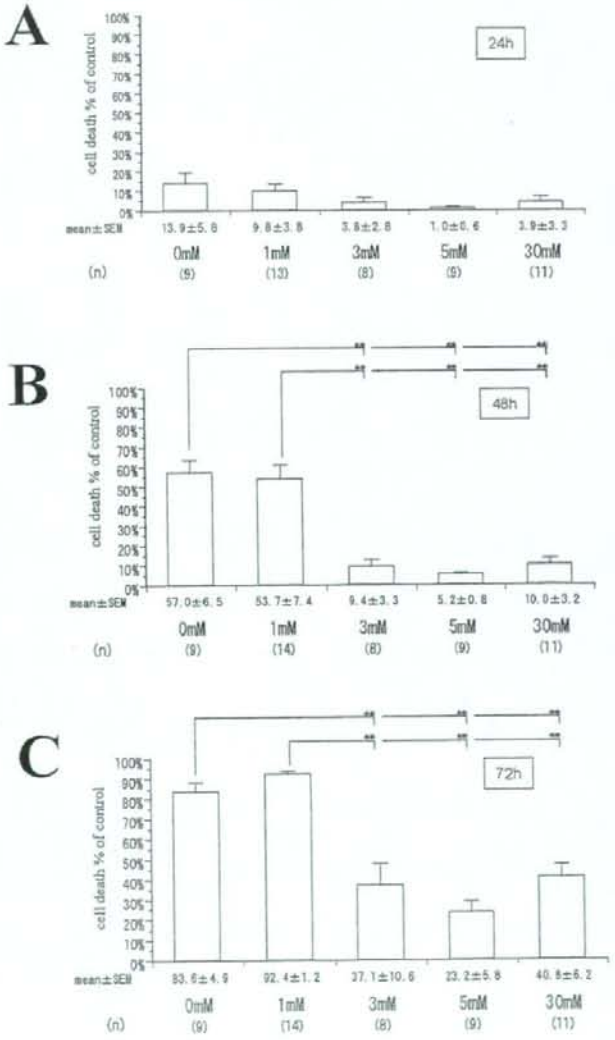

Fig. 2. The CA1 neuronal cell death during low glucose.

Various glucose concentrations (0mM, 1mM, 3mM, 5mM, and 30mM) under the environment lacking serum on the CA1 pyramidal cell. Glucose 0mM (n=9) and 1mM (n=14) resulted prominently high cell death rates after 48 hour culture and the cell death rates were 57.0±6.5% and 53.7±7.4%, respectively. After 72 hour, the rate further increased and the each rate was 83.6± 4.9% and 92.9±1.2%, respectively. The cell death rates showed the U-shaped curve against the glucose concentrations and neuronal cell death was minimum at 5mM glucose (n=9) (Fig. 2) (*:One-way ANOVA; $P=0.0008$, Scheffe's F test; $p<0.05$) (**:One-way ANOVA; $P<0.0001$, Scheffe's F test; $p<0.01$).

The DG granule cell death during low glucose

The difference in cell death rates was examined in various glucose concentrateions (0mM, 1mM, 3mM, 5mM, and 30mM) under

the environment lacking serum on the DG granule cell. The cell death rates increased maximally at 0mM glucose and the cell death rate after 48 hours was 59.2±5.3%. The DG granule cell was kept relatively well alive even in 1mM glucose condition and the death rate after 48 hours was only 14.5 ± 5.0%. The cell death rates did not show the U-shaped curve against the glucose concentrations and neuronal cell death was most inhibited at 3mM glucose (one-way ANOVA; $p<0.0001$, Scheffe's F test; $p<0.01$) (Fig. 3).

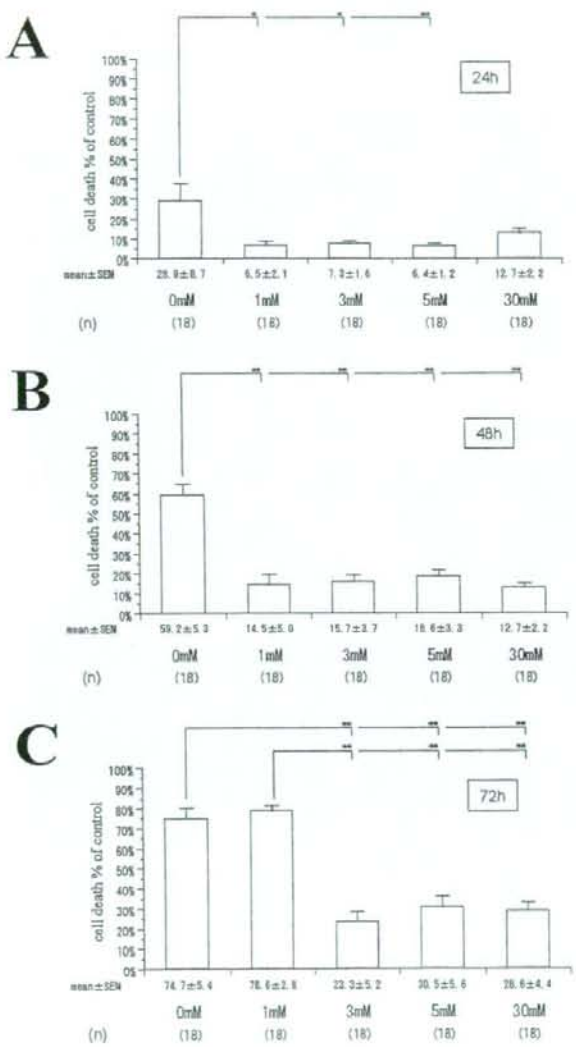

Fig. 3. The DG granule cell death during low glucose.

Various glucose concentrations (0mM, 1mM, 3mM, 5mM, and 30mM) under the environment lacking serum on the DG granule cell. The cell death rates increased maximally at 0mM glucose and the cell death rate after 48 hours was 59.2±5.3%. The DG granule cell was kept relatively well alive even in 1mM glucose condition and the death rate after 48 hours was only 14.5 ± 5.0%. The cell death rates did not show the U-shaped curve against the glucose concentrationns and neuronal cell death was most inhibited at 3mM glucose (one-way ANOVA; $p<0.0001$, Scheffe's F test; $p<0.01$) (Fig. 3).

CA1 neuronal cell death in the presence or absence of insulin

The difference in the cell death rates on the CA1 pyramidal neuron was examined in the presence or absence of 1nM insulin during treatment with a variety of glucose concentrations (0mM, 3mM, and 30mM). In the presence or absence of insulin at 3mM glucose, the observed cell death rates after 48 hours were 10.3±1.2% and 38.1±9.1%, respectively, and after 72 hours were 22.4±3.8% and 54.5±8.2%, respectively ($p<0.05$) (Fig.4B). Thus, the presence of insulin significantly improved the cell survival at 3mM glucose concentrations up to 72hours. In the case of 0mM glucose, the insulin addition surprisingly deteriorated the cell survival and the cell death rates of the presence or absence of insulin were 67.0 ± 10.5% and 37.3 ± 8.9%, respectively ($p<0.05$) (Fig. 4A). At 30mM glucose condition, the insulin addition did not give any significant effects on the cell survival ($p=0.789$) (Fig. 4C).

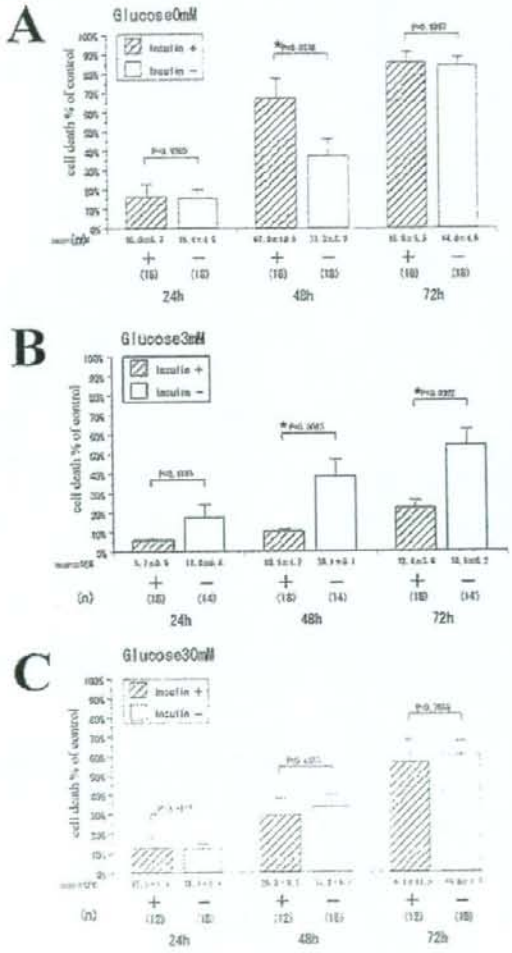

Fig. 4. CA1 neuronal cell death in the presence or absence of insulin.

The presence or absence of 1nM insulin during treatment with a variety of glucose concentrations (0mM, 3mM, and 30mM). In the presence or absence of insulin at 3mM glucose, the observed cell death rates after 48 hours were 10.3±1.2% and 38.1±9.1%, respectively, and after 72 hours were 22.4±3.8% and 54.5±8.2%, respectively (*: non-paired t-test; $p<0.05$) (Fig.4B). Thus, the presence of insulin significantly improved the cell survival at 3mM glucose concentrations up to 72hours. In the case of 0mM glucose, the insulin addition surprisingly deteriorated the cell survival and the cell death rates of the presence or absence of insulin were 67.0 ± 10.5% and 37.3 ± 8.9%, respectively (*: non-paired t-test; $p<0.05$) (Fig. 4A). At 30mM glucose condition, the insulin addition did not give any significant effects on the cell survival (non-paired t-test; $p=0.789$) (Fig. 4C).

The DG granule cell death in the presence or absence of insulin

The difference in cell death rates on the DG granule cell was examined in the presence or absence of 1nM insulin. The presence and absence of insulin at 3mM glucose showed no significant difference after 48 hours (22.1± 3.2 % and 25.5±3.6%, respectively) (p=0.4963). After 72 hours, the insulin showed minor protective effect against the cell death (31.8 ± 4.2% and 46.6 ± 4.1%, respectively) (p=0.0161). At 0mM glucose, the insulin addition deteriorated the cell survival and the cell death rates after 48 hours in the presence or absence of insulin were 47.2 ± 9.2% and 23.9 ± 3.7%, respectively (p<0.05) (Fig. 5A) . At 30mM glucose condition, no significant difference was observed between the insulin and non-insulin groups (p=0.2074) (Fig. 5C).

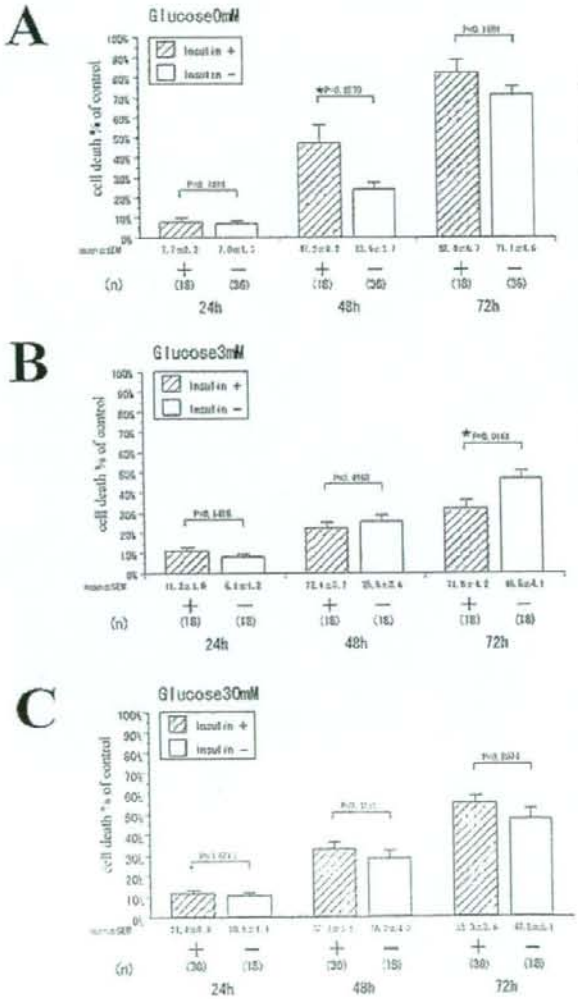

Fig. 5. The DG granule cell death in the presence or absence of insulin.

The presence or absence of 1nM insulin. The presence and absence of insulin at 3mM glucose showed no significant difference after 48 hours (22.1± 3.2 % and 25.5±3.6%, respectively) (non-paired t-test; p=0.4963). After 72 hours, the insulin showed minor protective effect against the cell death (31.8 ± 4.2% and 46.6 ± 4.1%, respectively) (*: non-paired t-test; p=0.0161). At 0mM glucose, the insulin addition deteriorated the cell survival and the cell death rates after 48 hours in the presence or absence of insulin were 47.2 ± 9.2% and 23.9 ± 3.7%, respectively (*: non-paired t-test; p<0.05) (Fig. 5A) . At 30mM glucose condition, no significant difference was observed between the insulin and non-insulin groups (non-paired t-test; p=0.2074) (Fig. 5C).

DISCUSSION

In the present experiment, we examined the insulin effects on the neuronal cell death during low glucose condition, and found that the insulin protected neuronal cell with low glucose, but increased neuronal cell death in case of glucose free condition (Fig. 4A, Fig. 5A). Moreover, while the DG had more tolerant against low glucose, the neuroprotective effect of insulin during low glucose had more prominent on the CA1 than the DG.

Neurotrophic effects of serum

When serum is contained, a low neuronal cell death rate was observed in comparison with addition of insulin only (1nM), suggesting that a variety of factors contained in serum work for neuronal cell protection in addition to insulin (Fig. 1). Neurotrophic factors such as NGF (nerve growth factor) or BDNF (brain derived neurotrophic factor), and vitamin B family in serum are supposed to restrain apoptosis and promote neuronal survival (18, 27). Our results were coincident to these previous results.

The CA1 vulnerability to hypoglycemia

Selective vulnerability has been well known to date and particularly the CA1 is one of the most vulnerable sites in the CNS against a stress. It is commonly believed that glutamate excitotoxicity relates to selective vulnerability. Glutamate level in the hippocampus of mouse after ischemic stress was greater in the CA1 than that of the DG (6, 14, 25). Also it has been known that the extracellular glutamate level rises in a glucose-free condition (24). As for the functional selectivity, the field potential was reported to be well maintained with low glucose concentration on the DG compared with the CA1. Li et al explained these selectivity as the differential activity of phosphofructokinase (PFK), the key enzyme for glycolysis. And the DG, indeed, has a high PFK activity than that of the CA1 (10). We found that the DG showed more tolerance to hypoglycemia than the CA1 at 1mM glucose concentration (Fig. 2, Fig.3), indicating the lower dependency of the DG granule neurons on glucose for their survival. Albeit it is a well-known phenomenon, the precise mechanism of the difference of glucose sensitivity between the CA1 and the DG neurons will need more exploration.

The protective effect of insulin during low glucose

The culture medium was adjusted to prepare insulin at 1nM concentration. This concentration corresponds to a blood insulin level following a hypodermic injection of about 27 units of insulin as a conversion to a 50 kg human body (8). This amount is nearly equal to that used in a clinical treatment. There is a report on the experiment in that 4nM insulin successfully worked for the suppression of cell apoptosis in the CNS (23). Our results showed even the smaller dose of insulin could affect the neuronal cell death. In CA1, 3mM glucose with insulin treatment inhibited prominently the neuronal cell death (Fig. 4B). A question arose whether lower than 3mM glucose concentration with insulin might alter the cell death rate. We conducted the experiment at the condition of 1.5mM and 2mM glucose, and obtained an advantageous result for survival of the neuronal cell (data not show). In case of the DG, the insulin treatment did not inhibit the neuronal cell death (Fig. 5B, Fig. 5C). Insulin takes glucose actively in the cell through the GLUT4 translocation to convert ca. 50% of glucose to energy. Furthermore, insulin activates MAP kinase (mitogen-activated protein kinase) working as a cell propagation factor to support the neuronal cell (12). In addition, insulin induces the expression of BDNF (29). A cooperation of these factors takes probably an important role for survival of neurons. In the present study, the prominent inhibition of the neuronal cell death was found only in the CA1. The levels of mRNA of GLUT4 were found to express in higher degree in the CA1 than the DG (2). Therefore, the CA1 neurons will be affected more influence by insulin than the DG neurons, at least on

glucose transport. Moreover, it has been reported that the depression of the insulin signal in the CNS increases GSK activity (Glycogen Synthase Kinase-3), that may lead to induce the neuronal cell death (1). GSK-3 expresses more in the CA1 pyramidal neurons and these preference in the CA1 may a least partially explain the insulin-sensitive selective vulnerability of the CA1 (4).

The acceleration of cell death by insulin during glucose deprivation

It has long been alleged that a possible secondary action of insulin includes affecting an amino acid metabolism and a lipid metabolism to enhance protein synthesis and lipid synthesis resulting in inhibiting the use of a substrate other than glucose by the cell. Thus, the environment lacking an enough amount of glucose may allow insulin to work negatively for cell survival (7). AMPK (AMP-activated protein kinase) that enhances the glucose transportation in a hypoxia tissue, is reduced by insulin treatment in an ischemic heart muscle (3, 5, 9, 19, 28). It is possible that insulin may block the induction of AMPK during glucose deprivation, and thus result in increase of cell death. Interestingly, an in vivo experiment reported that the neuronal protection effect of insulin showed the U-shaped curve, having a maximum peak in 6 to 7mM of the glucose level, and insulin rather accelerated the neuronal cell death at 2 to 3mM or the lower concentration of glucose (30).

CONCLUSION

Insulin therapy is now a common strategy for diabetic treatment, and caution for its therapy has been paid mainly on the treatment related hypoglycemia. Our study indicated that in central nervous system, insulin indeed has double-edged effects, and while neuronal survival is promoted in the presence of the adequate concentrations of glucose, the hazard effect of hypoglycemia may be accelerated by the presence of insulin. The selective vulnerability did not exist in this hypoglycemia-related insulin neuronal toxicity. The further study for this mechanism especially on the molecular cascade may lead to the better clinical management for diabetic care particularly on the prevention of the CNS complication.

REFERENCE

1. **Bhat RV, Budd Haeberlein SL, Avila J.** 2004. Glycogen synthase kinase 3: a drug target for CNS therapies. J Neurochem. **89(6)**: 1313-7.
2. **El Messari S., Aït-Ikhlef A., Ambroise DH., Penicaud L., Arluison M.** 2002. Expression of insulin-responsive glucose transporter GLUT4 mRNA in the rat brain and spinal cord: an in situ hybridization study.J Chem Neuroanat.**24(4)**: 225-42.
3. **Gamble J, Lopaschuk GD.** 1997. Insulin inhibition of 5' adenosine monophosphate-activated protein kinase in the heart results in activation of acetyl coenzyme A carboxylase and inhibition of fatty acid oxidation. Metabolism. **46(11)**: 1270-4.
4. **Hanger DP, Hughes K, Woodgett JR, Brion JP, Anderton BH. 1992.** Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. Neurosci Lett. **147(1)**: 58-62.
5. **Hayashi T, Hirshman MF, Kurth EJ, Winder WW, Goodyear LJ.** 1998. Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. Diabetes. **47(8)**: 1369-73.

6. **Heath PR, Shaw PJ.** 2002. Update on the glutamatergic neurotransmitter system and the role of excitotoxicity in amyotrophic lateral sclerosis. Muscle Nerve. **26(4)**: 438-58.
7. **Hellerstein MH, Munro HN.** 1994. Interaction of liver, muscle, and adipose tissue in the regulation of metabolism in response to nutritional and other factors. p.1169-1191, The Liver : Biology and Pathobiology 3rd ed (Arias IM, Boyer JL, Fausto N et al eds), Raven Press, New York.
8. **Kosaka K. et al.** 1988. Blood insulin level after the subcutaneous injection. The Latest Medicine (in Japanese). **43(3)**: 590.
9. **Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW.** 1999. 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes. **48(8)**: 1667-71.
10. **Li X, Yokono K, Okada Y.** 2000. Phosphofructokinase, a glycolytic regulatory enzyme has a crucial role for maintenance of synaptic activity in guinea pig hippocampal slices. Neurosci Lett. **294(2)**: 81-4.
11. **Macklis, J.D. and R.D. Madison.** 1990. Progressive incorporation of propidium iodide in cultured mouse neurons correlates with declining electrophysiological status: a fluorescence scale of membrane integrity. J. Neurosci. Methods. **31**: 43-46.
12. **Marshall CJ.** 1995. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell. **80(2)**: 179-85.
13. **Martin DJ, Warren LA, Gunatillake PA, McCarthy SJ, Meijs GF, Schindhelm K.** 2001. New methods for the assessment of in vitro and in vivo stress cracking in biomedical polyurethanes. Biomaterials. **22(9)**: 973-8.
14. **Nakamura N, Negishi K, Hirano A, Sugawara M. 2005.** Real-time monitoring of L-glutamate release from mouse brain slices under ischemia with a glass capillary-based enzyme electrode. Anal Bioanal Chem. **383(4)**: 660-7.
15. No authors listed. 1987. Diabetes Control and Complications Trial (DCCT): results of feasibility study. The DCCT Research Group. Diabetes Care. **10(1)**: 1-19.
16. **Northcutt RG.** 1989. Body and Brain. A Trophic Theory of Neural Connections. Science. **244(4907)**: 993.
17. **Ott A., Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM.** 1999. Diabetes mellitus and the risk of dementia: The Rotterdam Study.Neurology. **53(9)**: 1937-42.
18. **Putcha GV, Moulder KL, Golden JP, Bouillet P, Adams JA, Strasser A, Johnson EM.** 2001. Induction of BIM, a proapoptotic BH3-only BCL-2 family member, is critical for neuronal apoptosis. Neuron. **29(3)**: 615-28.
19. **Russell RR 3rd, Bergeron R, Shulman GI, Young LH.** 1999. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. Am J Physiol. **277(2 Pt 2)**: H643-9.
20. **Sakaguchi T, Okada M, and Kawasaki K.** 1994. Sprouting of CA3 pyramidal neurons to the dentate gyrus in rat hippocampal organotypic cultures. Neurosci. Res. **20**: 157-164.
21. **Saltiel AR, Kahn CR.** 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. **414(6865)**: 799-806.
22. **Saltiel AR, Pessin JE.** 2002. Insulin signaling pathways in time and space. Trends Cell Biol. **12(2)**: 65-71.
23. **Sima AA, Kamiya H, Li ZG.** 2004. Insulin, C-peptide, hyperglycemia, and central nervous system complications in diabetes. Eur J Pharmacol. **490(1-3)**: 187-97.

24. **Takata T, Hirai H, Shigemoto T, Okada Y.** 1995. The release of glutamate and accumulation of intracellular calcium in the guinea pig hippocampal slices during glucose deprivation. Neurosci Lett. **189(1)**: 21-4.
25. **Tomiyama M, Kimura T, Maeda T, Tanaka H, Furusawa K, Kurahashi K, Matsunaga M.** 2001. Expression of metabotropic glutamate receptor mRNAs in the human spinal cord: implications for selective vulnerability of spinal motor neurons in amyotrophic lateral sclerosis. J Neurol Sci. **189(1-2)**: 65-9.
26. **Voll CL, Auer RN.** 1991. Insulin attenuates ischemic brain damage independent of its hypoglycemic effect. J Cereb Blood Flow Metab. **11(6)**: 1006-14.
27. **Whitfield J, Neame SJ, Paquet L, Bernard O, Ham J.** 2001. Dominant-negative c-Jun promotes neuronal survival by reducing BIM expression and inhibiting mitochondrial cytochrome c release. Neuron. **29(3)**: 629-43.
28. **Witters LA, Kemp BE.** 1992. Insulin activation of acetyl-CoA carboxylase accompanied by inhibition of the 5'-AMP-activated protein kinase. J Biol Chem. **267(5)**: 2864-7.
29. **Yasuda M, Fukuchi M, Tabuchi A, Kawahara M, Tsuneki H, Azuma Y, Chiba Y, Tsuda M.** 2007. Robust stimulation of TrkB induces delayed increases in BDNF and Arc mRNA expressions in cultured rat cortical neurons via distinct mechanisms. J Neurochem. **103(2)**: 626-36.
30. **Zhu CZ, Auer RN.** 2004. Optimal blood glucose levels while using insulin to minimize the size of infarction in focal cerebral ischemia. J Neurosurg. **101(4)**: 664-8.

66巻増刊号1(通巻929号)・2008年1月28日発行　ISSN 0047-1852 Nippon Rinsho

日本臨牀 増刊号

アルツハイマー病

基礎研究から予防・治療の新しいパラダイム

株式会社 日本臨牀社

アルツハイマー病の新しい治療法の開発
新規医薬品

チアゾリジン誘導体

Thiazolidine derivatives

櫻井 孝　横野浩一

Key words：チアゾリジン誘導体，アルツハイマー病，糖尿病

はじめに

チアゾリジン誘導体(TZD)はインスリン抵抗性改善薬であり，世界的にはpioglitazone (Actos)とrosiglitazone(Avandil)の2種類が発売されており，我が国では前者のみが承認されている．TZDはブドウ糖・脂質代謝を改善するのみではなく，抗炎症作用，抗動脈硬化作用が報告されており，最近ではアルツハイマー病(AD)の治療薬としても期待されている．一方，TZDには副作用として，浮腫，体重増加，心不全があり，その使用にあたっては慎重な管理を要する．今日，我が国は超高齢社会にあり，後期高齢者になって寝たきりとなる原病をたどるとその多くは生活習慣病である．サクセスフルエイジングのためにもTZDを有効に利用し，生活習慣病や認知症の進展を抑制することが重要である．

本稿では特にAD治療薬としてのTZDについて，最近の知見をまとめ紹介したい．

1. チアゾリジンの作用機序

ペルオキシゾーム増殖因子活性化受容体(peroxisome proliferators-activated receptor: PPAR)は，核内受容体スーパーファミリーの一つで，体内および食品に存在する低分子量の脂溶性生理活性物質(15-deoxy $\Delta^{12,14}$ prostaglandin J2など)をリガンドとしている．PPARsは主に糖・脂質代謝にかかわる遺伝子群の発現制御を行い，PPARにはα，β/δ，γの3つのタイプが同定されている．PPARαアゴニストであるフィブラート系薬剤は高脂血症治療薬として，PPARγアゴニストであるTZDは糖尿病治療薬として臨床応用されている．PPARγには更に2つのアイソフォームがあり，脂肪組織にPPARγ2が，マクロファージおよび血管にPPARγ1が存在する．PPARγ2は脂肪細胞の分化を促進し，糖取り込みやインスリン感受性にかかわる分子の発現を亢進させる．また同時にアディポネクチンの発現を亢進させる．PPARγ1はNF-κBとダイマーを形成し，その作用を阻害することで，MCP-1，VACM-1，TNF-α，COX-2，CRPなどの炎症性マーカーを抑制する[1]．

脳ではPPARγは，神経細胞，グリア細胞および脳血管に存在する．TZDはPPARγ活性化により炎症物質の発現を抑制する．神経変性疾患，虚血を問わず，炎症は神経細胞死を誘導する因子であり，このためPPARγはADのみならず，グルタミン酸による神経障害，脳虚血，パ

Takashi Sakurai, Koichi Yokono：Department of Geriatric Medicine, Kobe University Graduate School of Medicine　神戸大学大学院医学系研究科 老年内科

0047-1852/08/¥40/頁/JCLS

ーキンソン病，多発性硬化症などの疾患でも，治療の新たなターゲットとして期待されている．

2. 糖尿病とアルツハイマー病

糖尿病では非糖尿病に比して認知機能障害が認められる．Strachanらの総説では，注意-集中力の低下，前頭葉-遂行機能の障害，視覚性記憶また言語性の記憶低下，精神運動性知能の低下，一般的な知能検査の成績が低下している[2]．しかし糖尿病にみられる認知機能低下は，日常生活に支障を来す程度のものではないと考えられ，これまで多くの関心を集めることはなかった．

ところが近年の疫学調査により，高齢者糖尿病では認知症との合併が多いことが明らかになってきた．糖尿病におけるAD，血管性認知症の相対危険度は，各々1.3-2.3倍，2.0-3.5倍程度とされる[3]．最も信頼性の高い研究とされるRotterdam研究では，インスリン使用者で認知症の相対危険度が4.3倍と高いことが報告された[4]．Honolulu-Asia研究では，ADの遺伝的危険因子であるApo E ε4を保持する高齢者2型糖尿病で相対危険度は更に高いこと，また海馬に老人斑，神経原線維変化が出現すると相対危険度が2.5-3倍高値であることが示され，これまでの臨床解析を裏づける病理成績が示された[5]．現在，どのような高齢者糖尿病で認知機能が低下し，認知症の発症が多いかについて，世界中で研究が進められている．糖尿病の血管性，代謝性要因の中でも，高インスリン血症はADの発症機構の根幹にかかわる可能性があり，以下に述べたい．

3. アルツハイマー病における高インスリン血症の関与

インスリンは，脳血管関門を通過し，海馬，大脳皮質，視床下部などに分布するインスリン受容体に結合する．脳内でもインスリンは少量産生される．高インスリン血症では，脳のインスリン取り込みがdown-regulationを受け，長期的には脳内のインスリンシグナルが低下する可能性が提唱されている[6,7]．実際，ADではインスリン受容体が増加しており，インスリン受容体以降のシグナルであるチロシンキナーゼ活性が低下している．

脳においてインスリンは糖エネルギー代謝を調整するばかりではなく，アセチルコリンやノルエピネフリンなどの神経伝達物質の合成を調節し，シナプスの可塑性，記憶や学習に深くかかわる[7]．

一方，インスリンはアミロイドβやタウの代謝にも作用する．インスリンは神経細胞内のアミロイドβの細胞外への分泌を刺激し，またアミロイドβの消化酵素の一つであるインスリン分解酵素(insulin degrading enzyme: IDE)の発現を調整している．ADでは海馬でのIDEの発現が低下しており[8]，IDE関連遺伝子の異常も指摘されている．このため脳内インスリン作用が低下すると，アミロイドβの分解が低下し，アミロイドβの神経細胞内での蓄積が促進される[7]．また，脳から末梢循環中に排出されたアミロイドβのクリアランスが，高インスリン血症により低下する可能性も指摘されている．

更に高インスリン血症では炎症が惹起され，脳脊髄液中のIL-1β，IL-6，TNF-αが増加していることが報告されている[7]．これらの作用を介して高インスリン血症は脳機能を低下させ，AD発症のリスクになると考えられている(図1)．最近ではADにおける脳のインスリン作用不足に伴う代謝異常を3型糖尿病と呼ぶ論文もみられる[9]．

4. アルツハイマー病のチアゾリジンによる治療

ADの予防に消炎鎮痛薬(NSAIDs)が有効である可能性が以前より指摘されていたが，NSAIDsのターゲットがPPARγの活性化にあることが明らかとなった[10]．この発見を契機に，また上述のADと高インスリンの関連から，TZDがAD治療に有効であることが期待されている．TZDが炎症性サイトカインを抑制し，アミロイド前駆体蛋白の代謝酵素であるBACE1の発現を軽減させること，アミロイドβの脳内でのクリアランスを亢進させること，またADモデル

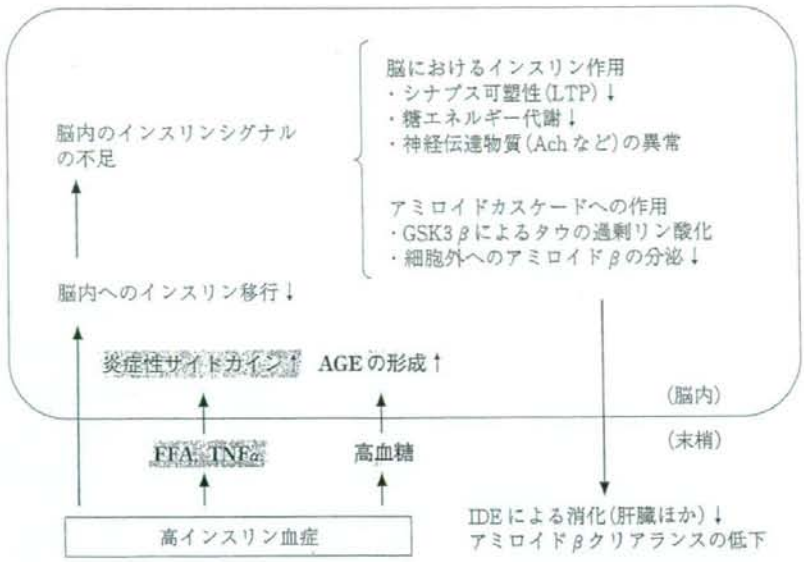

図1 高インスリン血症とアルツハイマー病

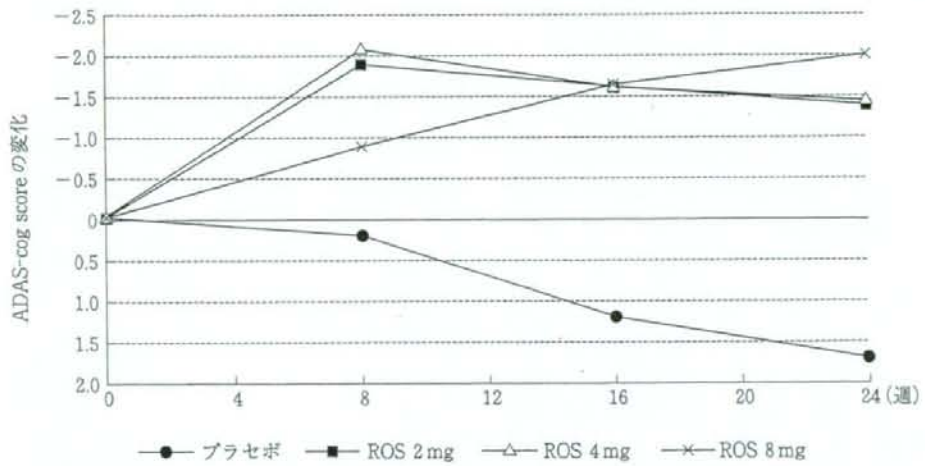

図2 アルツハイマー病に対するロシグリタゾンの効果
Apo E ε4非保持者のみ表示.（文献[11]より改変）

動物で経口投与されたTZDがアミロイドβの蓄積を減少させるなど，基礎的データが蓄積されつつある[10].

最近，軽症のADやamnestic MCIを対象として，rosiglitazoneとプラセボの無作為二重盲検試験が報告された[11]．その結果，全体解析では両群間に差はみられなかったが，Apo E ε4非保持者ではrosiglitazone投与群で，記憶や注意力が改善していた(図2)．Apo E ε4非保持者のADでは，脳脊髄液/血液インスリン比が低値であるという[7]．またrosiglitazone，pioglitazoneは，少量であるが脳へ移行するとの報告もみられ[12]，TZDは高インスリン血症改善による作用，抗炎症作用または直接作用により脳機能を改善したものと考えられる．現在，TZDはADのモデュレーターとして治療の選択肢に考えられつ

つあり，米国ではrosiglitazoneを用いた第三相臨床試験が進行している．

5. 投与上の注意とまとめ

TZDの副作用として浮腫は重要であり，心不全を惹起するとの報告もある．2003年にはAHAとADAによる共同声明が[13]，2007年にはrosiglitazoneによる心筋梗塞リスクが記載された[14]．ここでは我が国におけるpioglitazoneの市販後調査であるPRACTICAL(糖尿病を対象)の結果を基に説明する[15]．

TZDが浮腫を来す機序として，PPARγ刺激を介した腎尿細管ナトリウム再吸収の促進作用が知られている．浮腫の発現頻度は男性4.2%，女性で12.1%であり，女性に多く，用量依存性に増加する．浮腫のリスクとして，①女性，②糖尿病合併症あり，③糖尿病罹病期間が長い，④高血圧の合併，⑤BMI高値，⑥高齢者があげられる．一方，心不全に進行するリスク要因としては，心不全・心筋梗塞・冠動脈疾患の既往，高血圧，左室肥大，弁膜症，高齢者，糖尿病罹病歴10年以上，インスリンの併用，慢性腎疾患(クレアチニン2.0mg/dl以上)などがある．よって，基本姿勢として投与前後に胸部X線，心エコー，BNP値で心不全・体液貯留をチェックすることが重要である．集合尿細管におけるナトリウムチャネル阻害薬であるトリアムテレン(カリウム保持性利尿薬)が浮腫に有効と考えられるが，実際にはループ利尿薬の使用による浮腫のコントロールが一般的である．

一方，pioglitazoneは心血管障害・脳卒中の予防に有効であることが既に証明されている(PROactive)．同じPPARγ作動薬でありながらrosiglitazoneとpioglitazoneとの差異については不明な点も残るが，現状では慎重な投与が望まれる．

■文　献

1) 門脇　孝：ピオグリタゾンの心血管保護作用．日本臨牀　64：156-159，2006.
2) Strachan MW, et al: Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. Diabetes Care 20: 438-445, 1997.
3) Biessels GJ, et al: Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol 5: 64-74, 2006.
4) Ott A: Diabetes mellitus and the risk of dementia: The Rotterdam Study. Neurology 53: 1937-1942, 1999.
5) Peila R, et al: Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. Diabetes 51: 1256-1262, 2002.
6) Kim SP, et al: Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. Diabetes 52: 2453-2460, 2003.
7) Craft S: Insulin resistance syndrome and Alzheimer's disease: age- and obesity-related effects on memory, amyloid, and inflammation. Neurobiol Aging 26(Suppl 1): 65-69, 2005.
8) Gasparini L, et al: Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling. J Neurosci 21(8): 2561-2570, 2001.
9) de la Monte SM, et al: Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: relevance to Alzheimer's disease. J Alzheimers Dis 10: 89-109, 2006.
10) Sundararajan S, et al: PPARgamma as a therapeutic target in central nervous system diseases. Neurochem Int 49(2): 136-144, 2006.
11) Risner ME, et al: Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. Pharmacogenomics J 6: 246-254, 2006.
12) Strum JC: Rosiglitazone induces mitochondrial biogenesis in mouse brain. J Alzheimers Dis 11: 45-51, 2007.
13) Nesto RW, et al: Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. October 7, 2003. Circulation 108: 2941-2948, 2003.