Serum Level of Endogenous Secretory Receptor for Advanced Glycation End Products and Other Factors in Type 2 Diabetic Patients With Mild Cognitive Impairment

Gang Chen, MD1
Liangchun Cai, MD1
Bin Chen, MD2
Jixing Liang, MD1
Fenhuai Lin, MD2
Liantao Li, MD1
Lixiang Lin, MD1
Jin Yao, MD1
Junping Wen, MD1
Huibin Huang, MD1

OBJECTIVE—Determine the serum levels of endogenous secretory receptor for advanced glycation end products (esRAGEs) in patients with type 2 diabetes and mild cognitive impairment (MCI) and in control patients with type 2 diabetes but no MCI, and examine the relationship of esRAGE and MCI with other clinical factors.

RESEARCH DESIGN AND METHODS—A total of 101 patients with type 2 diabetes who were hospitalized in the Department of Endocrinology at Fujian Provincial Hospital between January 2010 and January 2011 were enrolled. There were 58 patients with MCI and 43 patients without MCI (control). Serum levels of esRAGE were measured using an enzyme-linked immunosorbent assay (ELISA). Other clinical parameters were also measured.

RESULTS—Type 2 diabetic patients with MCI had a longer duration of diabetes; elevated HbA1c, total cholesterol (CHOL), LDL cholesterol (LDL-C), triglyceride (TG), intima-media thickness, C-reactive protein (CRP), and brachial-ankle pulse wave velocity (ba-PWV), and lower ankle brachial index (ABI) and esRAGE relative to the control group. Among patients with MCI, the Montreal Cognitive Assessment (MoCA) score was positively correlated with serum esRAGE but negatively correlated with CHOL. Spearman rank correlation analysis indicated that esRAGE was positively correlated with MoCA score and ABI but negatively correlated with ba-PWV, CHOL, TG, and CRP in all subjects.

CONCLUSIONS—Our results suggest that esRAGE may be a potential protective factor for dyslipidemia, atherosclerosis, and MCI in patients with type 2 diabetes.

Diabetes Care 34:2586–2590, 2011
Serum samples were isolated from fasting and other clinical parameters. Measurement of esRAGE, AGEs, and other clinical parameters

Serum samples were isolated from fasting subjects and stored at −80°C prior to analysis. The serum levels of esRAGE and AGE were measured by ELISA kits from R&D Systems (Minneapolis, MN). For esRAGE, the intra-assay coefficient of variation (CV) was <10% and the interassay CV was <4%; for AGEs, the intra-assay CV was 5.8% and the interassay CV was 9.8%. Blood lipids, C-reactive protein (CRP), urine microalbumin, and HbA1c levels were quantified using an automatic biochemical analyzer (Bio-Rad, Benicia, CA). Waist circumference, height, and body weight were measured, and BMI (kg/m²) was calculated.

Measurement of atherosclerosis

A VP 1000 automated atherosclerosis analyzer (Colin Medical Technology Corp., Komaki, Japan) was used to measure brachial-ankle pulse wave velocity (ba-PWV) and ankle brachial index (ABI). The PWV between two recording sites is related to arterial wall distensibility, so the severity of atherosclerosis can be determined by vessel evaluation and wave analysis. The blood pressure cuffs were tied at both elbows and ankles to measure ba-PWV and ABI. The higher of the two ba-PWV values and the lower of the two ABI values were used for statistical analysis.

Intima-media thickness determination

Color Doppler ultrasound was used to locate the thickest site of the carotid artery intima-media and to measure the thickness at this site, two other upstream sites, and one site that was 1 cm downstream, each of which were measured six times to obtain average values.

Statistical analyses

All data and parameters are presented as means and SDs and were analyzed using SPSS version 13.0. Data with normal and homoscedastic distributions were analyzed by Student t test, and those with nonnormal distribution or heteroscedastic distributions were analyzed by the Wilcoxon rank-sum test. The χ² test was used to compare the incidences of certain events, such as smoking. The factors influencing MoCA score were analyzed using Pearson correlation analysis for normally distributed variables and Spearman rank correlation for nonnormally distributed variables. The factors influencing serum esRAGE were analyzed by Spearman rank correlation analysis. A P value of 0.05 was considered significant.

RESULTS

Demographic and clinical characteristics of enrolled patients

Table 1 shows the demographic and clinical characteristics of enrolled type 2 diabetic patients with and without MCI. There were no significant differences between the groups in age, sex, BMI, waist circumference, morning urinary microalbumin, HDL cholesterol (HDL-C), duration of hypertension, and history of smoking (P > 0.05). Compared with the control group, patients with MCI had a significantly longer duration of diabetes; elevated total cholesterol (CHOL), LDL cholesterol (LDL-C), triglyceride (TG), CRP, intima-media thickness (IMT), and ba-PWV; and lower ABI (P < 0.01 for all). HbA1c was also higher in the MCI group (P < 0.05). The serum esRAGE level was significantly lower in the MCI group (P < 0.01), and the serum AGE level was significantly lower in the control group (P < 0.05) (Table 1).

We also assessed the use of medications by all enrolled patients (Table 2). The results indicate no significant differences in medication use between the two groups.

Correlation of MoCA scores and other clinical indicators

Next, we assessed the association of MoCA scores with different variables by use of Pearson correlation analysis for normally distributed variables and Spearman rank correlation analysis for nonnormally distributed variables (Table 3). The results indicated that MoCA score was positively correlated with serum esRAGE level (r = 0.942), but negatively correlated with CHOL (r = −0.364) and AGEs (r = −0.275) (Table 3). MoCA was not significantly correlated with patient age, duration of diabetes, HbA1c, BMI, waist circumference, blood lipids, CRP, IMT, ba-PWV, or ABI (P > 0.05 for all).

Correlation of esRAGE and other clinical indicators

Finally, we performed Spearman rank correlation analysis of esRAGE (dependent variable) and other clinical indicators (independent variables). Multiple linear regression analysis was performed to determine the factors that influence serum esRAGE level. The results indicated that esRAGE was positively correlated with age, duration of diabetes, waist circumference, and CRP (Table 4). The factors influencing serum AGE level were analyzed using Pearson correlation analysis, and the results indicated that AGE was positively correlated with BMI, waist circumference, CRP, and duration of diabetes (Table 5).

Table 1—Demographic and clinical characteristics of type 2 diabetic patients with and without MCI

|                  | Type 2 diabetes with MCI | Type 2 diabetes without MCI (control) |
|------------------|-------------------------|---------------------------------------|
| Number of patients | 58                      | 43                                    |
| Male/female      | 30/28                   | 24/19                                 |
| Age (years)      | 63.90 ± 8.73            | 62.84 ± 7.94                          |
| Duration of type 2 diabetes (years) | 11.19 ± 4.73           | 5.19 ± 3.55**                         |
| BMI (kg/m²)      | 24.19 ± 2.85            | 23.07 ± 2.98                          |
| Waist circumference (cm) | 86.22 ± 9.08      | 84.61 ± 9.47                          |
| HbA1c (%)        | 9.35 ± 2.40             | 8.19 ± 1.94*                          |
| CHOL (mmol/L)    | 5.31 ± 1.36             | 4.52 ± 1.24**                         |
| LDL (mmol/L)     | 3.24 ± 1.01             | 2.57 ± 1.11**                         |
| TG (mmol/L)      | 2.05 ± 2.33             | 1.34 ± 1.05**                         |
| HDL (mmol/L)     | 1.15 ± 0.39             | 1.33 ± 0.52                           |
| IMT (mm)         | 1.19 ± 0.17             | 1.05 ± 0.19**                         |
| CRP (mg/L)       | 9.37 ± 14.12            | 2.54 ± 6.64**                         |
| ABI              | 1.09 ± 0.12             | 1.17 ± 0.08**                         |
| ba-PWV (cm/s)    | 2,090.76 ± 536.817     | 1,646.60 ± 423.626**                  |
| Morning urine microalbumin (mg/L) | 117.46 ± 411.68     | 89.91 ± 343.30                        |
| Duration of hypertension (years) | 7.44 ± 9.48          | 5.76 ± 8.45                           |
| Had ever smoked (%) | 18/58 (31.03%)        | 11/43 (25.58%)                        |
| Current smoking (%) | 13/58 (22.41%)       | 6/43 (13.95%)*                        |
| Habitual alcohol drinking (%) | 10/58 (17.24%)      | 7/43 (16.28%)                         |
| esRAGE (µg/L)    | 3.51 ± 2.91             | 7.82 ± 5.24**                         |
| AGEs (ng/L)      | 1,999.08 ± 1,207.48    | 1,377.08 ± 905.37*                    |

The Student t test, Wilcoxon rank-sum test, or χ² test was used to test for significant differences. *P < 0.05. **P < 0.01.
esRAGE and diabetes with mild cognitive impairment

Table 2—Comparison of drug use by type 2 diabetic patients with and without MCI

| Drug Category                      | Type 2 diabetes with MCI | Type 2 diabetes without MCI (control) |
|-----------------------------------|--------------------------|---------------------------------------|
| Number of patients                | 58                       | 43                                    |
| Insulin dose (units)              | 20.86 ± 17.48            | 19.81 ± 20.40                         |
| The use of insulin (%)            | 30/58 (67.24%)           | 26/43 (60.47%)                        |
| Oral hypoglycemic drugs (%)       | 36/58 (62.07%)           | 32/43 (74.42%)                        |
| Sullonlyures (%)                  | 11/58 (18.97%)           | 9/43 (20.93%)                         |
| Nateglinide or repaglinide (%)    | 8/58 (13.79%)            | 7/43 (16.28%)                         |
| Biguanides (%)                    | 29/58 (50.00%)           | 23/43 (53.49%)                        |
| Thiazolidinediones (%)            | 3/58 (5.17%)             | 2/43 (4.65%)                          |
| α-Glucosidase inhibitor (%)       | 23/58 (39.66%)           | 24/43 (55.81%)                        |
| Antihyperensive medications (%)   | 41/58 (70.69%)           | 25/43 (58.14%)                        |
| Diuretics (%)                     | 12/58 (20.69%)           | 6/43 (13.95%)                         |
| β-Blockers (%)                    | 11/58 (18.97%)           | 6/43 (13.95%)                         |
| Calcium channel blockers (%)      | 21/58 (36.21%)           | 19/43 (44.19%)                        |
| Angiotensin-converting enzyme inhibitors (%) | 14/58 (24.14%)       | 6/43 (13.95%)                         |
| Angiotensin II receptor blockers (%) | 17/58 (29.31%)         | 14/43 (32.56%)                        |
| α1-Blockers (%)                   | 5/58 (8.62%)             | 0/43 (0.00%)                          |
| Antiplatelet medications (%)      | 37/58 (63.79%)           | 23/43 (53.49%)                        |
| Lipid-lowering medications (%)    | 37/58 (63.79%)           | 27/43 (62.79%)                        |

The Student t test, Wilcoxon rank-sum test, or χ² test was used to test for significant differences. All P values were >0.05.

Table 3—Relationship of MoCA score with other clinical indicators in patients with type 2 diabetes and MCI

| Clinical Indicator | r   | P   |
|--------------------|-----|-----|
| MoCA score         | 0.942 | <0.001 |
| AGES               | -0.275 | 0.005 |
| CHOL               | -0.364 | 0.005 |

Table 4—Relationship of esRAGE with other clinical indicators in type 2 diabetic patients with and without MCI (n = 101)

| Clinical Indicator | r   | P   |
|--------------------|-----|-----|
| AGES               | -0.236 | 0.017 |
| MoCA score         | 0.803 | 0.000 |
| ABI                | 0.214 | 0.032 |
| ba-PWV             | -0.371 | 0.000 |
| CHOL               | -0.303 | 0.002 |
| TG                 | -0.274 | 0.006 |
| CRP                | -0.308 | 0.002 |

variable (independent variable) in all 101 patients. The results indicated that esRAGE was positively correlated with MoCA score and ABI (r = 0.803 and r = 0.214, respectively), but negatively correlated with ba-PWV, CHOL, TG, and CRP (r = -0.371, r = -0.303, r = -0.274, and r = -0.308, respectively) (Table 4). There was no significant correlation of esRAGE with age, duration of diabetes, HbA1c, BMI, waist circumference, HDL, LDL, or IMT (P > 0.05 for all).

CONCLUSIONS—Our study of patients with type 2 diabetes indicated that MCI was associated with duration of diabetes; elevated levels of HbA1c, CHOL, LDL, TG, IMT, CRP, ABI, ba-PWV, and esRAGE; and low levels of serum AGES. In agreement with our results, numerous other studies have also reported associations of diabetes with vascular brain damage (8), degenerative nerve disease (9), cognitive decline (10,11), and dementia or MCI (12). In addition, MCI is associated with the onset, longer duration, and greater severity of diabetes (13). Diabetic patients have increased levels of AGES, inflammation, and oxidative stress, all of which can affect blood supply to the brain. We observed an association between hyperglycemia and MCI perhaps because hyperglycemia itself causes denaturation of neurons responsible for cognitive function, or because hyperglycemia accelerates the progression of atherosclerosis, which leads to MCI (14).

In agreement with our results, other studies (15) reported that sustained hyperglycemia, as indicated by elevated HbA1c, is an independent risk factor for impairment of cognitive function. There may be several pathogenic mechanisms underlying this process, including accumulation of sorbitol and development of a hyperosmotic state in nerve cells that leads to edema and impaired brain function (16), insulin resistance syndrome (17), impaired insulin homeostasis in the brain (18), and/or hyperinsulinemia (19).

Our results indicate that type 2 diabetic patients with MCI had significantly elevated CRP and ba-PWV but lower ABI than the control group, indicating a correlation between atherosclerosis and type 2 diabetes–associated MCI. This is consistent with the research of Rafnsson et al. (20), who reported that individuals with peripheral arterial disease had worse cognitive function than healthy controls, and with the research of Hanon et al. (21), who reported a significant relationship of reduced cognitive function and arteriosclerosis (based on PWV) in 308 elderly patients.

Our results also indicate that type 2 diabetic patients with MCI had significantly higher CRP levels than controls, suggesting an association of inflammation and MCI. In agreement with this result, Xu et al. (22) reported that patients with higher serum CRP had lower mini-mental state examination scores and higher risk for development of AD. Other studies have also shown that AD patients have increased brain inflammation and that this leads to deposition of amyloid β-protein (23), formation of senile plaques, and neurofibrillary formation, resulting in damage or death of neurons. Inflammation also increases apolipoprotein E synthesis in stellate cells, which is associated with increased risk of AD (24).

Previous evidence indicates that RAGE has multiple roles in the development of cognitive dysfunction. RAGE appears to promote AD through a positive feedback mechanism with amyloid β-protein (25). Another study reported that low expression of esRAGE in the hippocampus was associated with AD (26). Thus, the mechanism by which AGES induce MCI in patients with type 2 diabetes may include one or more of the following: 1) AGES induce oxidative stress directly or by binding to specific receptors such as RAGE, leading to upregulation of nuclear factor-kB and its target genes, resulting in inflammation and neural damage; 2) AGES induce vascular endothelial dysfunction, and the increased permeability of blood vessels increases accumulation of AGES in the vascular wall and causes hardening; and...
3) AGEs activate microglial cells and damage the microtubular structure, leading to neuron dysfunction.

Recent research has shown that esRAGE counteracts the effects of inflammatory molecules. Dementia patients have lower levels of esRAGE than healthy controls (27). Ghidoni et al. (28) reported that serum esRAGE levels were significantly decreased in MCI patients and even lower in AD patients than in healthy controls. Similar to these reports, our results indicated that esRAGE levels were lower in patients with an MCI group than those with type 2 diabetes alone, suggesting that esRAGE may have a protective effect against diabetic MCI. While the precise mechanism of this relationship remains unclear, one or more of the following may be responsible: 1) chronic hyperglycemia in type 2 diabetic patients with MCI directly inhibits synthesis and secretion of esRAGE; 2) accumulated AGEs bind to esRAGE, which leads to increased clearance of esRAGE; and 3) inflammation, which has been implicated in MCI, can also reduce esRAGE synthesis.

Our results also indicated that the MoCA score was positively correlated with serum esRAGE \( (r = 0.942) \) and negatively correlated with CHOL \( (r = -0.364) \) in type 2 diabetes patients with MCI. Thus, measurement of esRAGE and CHOL can be helpful in the prediction and/or diagnosis of MCI in middle-aged and older populations. We also found that esRAGE was negatively correlated with CHOL and TG, consistent with previous studies (29). Thus, esRAGE may be an endogenous factor that protects against oxidative stress-mediated atherosclerosis and endothelial dysfunction in hypercholesterolemia (30).

Additionally, Lindsey et al. (31) reported that esRAGE was independently and negatively correlated with coronary atherosclerosis. On the other hand, there is some evidence that elevated esRAGE is associated with increased cardiovascular disease (32,33).

McNair et al. (34) reported that esRAGE was negatively correlated with high-sensitivity CRP (hs-CRP) and tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)), but that TNF-\( \alpha \) was positively correlated with hs-CRP. This result suggests that the production of esRAGE may be regulated by TNF-\( \alpha \)-related hs-CRP. Another study involving 245 type 2 diabetic patients without coronary artery disease shows negative correlation between esRAGE and hs-CRP (35). Consistent with these reports, we found that esRAGE was negatively associated with hs-CRP \( (r = -0.308) \).

In conclusion, our study indicates that a lower level of serum esRAGE and a higher level of serum AGE in patients with type 2 diabetes are associated with MCI, dyslipidemia, and atherosclerosis. Thus, we suggest that future investigators consider the hypothesis that therapeutic interventions that increase serum esRAGE may be a novel approach to prevent RAGE-mediated diseases such as MCI and AD.

Acknowledgments—This work was supported by grants C071002 and 2009Y0011 from the Natural Science Foundation of Fujian Province.

No potential conflicts of interest relevant to this article were reported.

G.C. designed the study and reviewed and edited the manuscript. L.C. wrote the manuscript and researched data. B.C. researched data and contributed to discussion. J.L., F.L., L. Li, L. Lin, J.Y., and J.W. researched data. H.H. reviewed and edited the manuscript.

References

1. Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2001;56:1133–1142.

2. Petersen RC, Roberts RO, Knopman DS, et al. Mild cognitive impairment: ten years later. Arch Neurol 2009;66:1447–1455.

3. Hubeckmann AG, Regensteiner JG, Vlassara H, Reusch JE. Diabetes and advanced glycation end products. Diabetes Care 2006;29:1420–1432.

4. Schmidt AM, Sahagan B, Nelson RB, Selmer J, Rothlein R, Bell JM. The role of RAGE in amyloid-beta peptide-mediated pathology in Alzheimer's disease. Curr Opin Investig Drugs 2009;10:672–680.

5. Santilli F, Vazzana N, Buicciarello LG, Davi G. Soluble forms of RAGE in human diseases: clinical and therapeutic implications. Curr Med Chem 2009;16:940–952.

6. Yan SF, Ramasamy R, Schmidt AM. Soluble RAGE: therapy and biomarker in unraveling the RAGE axis in chronic disease and aging. Biochem Pharmacol 2010;79:1379–1386.

7. Portet F, Ousset PJ, Visser PJ, et al.; MCI Working Group of the European Consortium on Alzheimer’s Disease (EADC). Mild cognitive impairment (MCI) in medical practice: a critical review of the concept and new diagnostic procedure. Report of the MCI Working Group of the European Consortium on Alzheimer’s Disease. J Neurol Neurosurg Psychiatry 2006;77:714–718.

8. Biessels GJ, van der Heide LP, Kamal A, Bleys RL, Gispert WH. Ageing and diabetes: implications for brain function. Eur J Pharmacol 2002;441:1–14.

9. Korf ES, White LR, Scheltens P, Launer LJ. Brain aging in very old men with type 2 diabetes: the Honolulu-Asia Aging Study. Diabetes Care 2006;29:2268–2274.

10. Gregg EW, Yaffe K, Cauley JA, et al. Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of Osteoporotic Fractures Research Group. Arch Intern Med 2000;160:174–180.

11. Logroscino G, Kang JH, Grodstein F. Prospective study of type 2 diabetes and cognitive decline in women aged 70–81 years. BMJ 2004;328:548.

12. Velayudhan L, Poppe M, Archer N, Proitsi P, Brown RG, Lovestone S. Risk of developing dementia in people with diabetes and mild cognitive impairment. Br J Psychiatry 2010;196:36–40.

13. Roberts RO, Geda YE, Knopman DS, et al. Association of duration and severity of diabetes mellitus with mild cognitive impairment. Arch Neurol 2008;65:1066–1073.

14. Fan WX, Parker R, Parpia B, et al. Erythrocyte fatty acids, plasma lipids, and cardiovascular disease in rural China. Am J Clin Nutr 1990;52:1027–1036.

15. Ryan CM, Geckle M. Why is learning and memory dysfunction in Type 2 diabetes limited to older adults? Diabetes Metab Res Rev 2000;16:308–315.

16. Pavlović DM, Pavlović AM. Dementia and diabetes mellitus. Srp Arh Celok Lek 2008;136:170–175 [in Serbian].

17. Panza F, Frisardi V, Seripa D, et al. Metabolic syndrome, mild cognitive impairment, and dementia. Curr Alzheimer Res 2011;8:492–509.

18. S Roriz-Filho J, S–Roriz TM, Rosset I, et al. (Pre)diabetes, brain aging, and cognition. Biochim Biophys Acta 2009;1792:432–443.

19. Young SE, Mainous AG 3rd, Carnemolla M. Hyperinsulinemia and cognitive decline in a middle-aged cohort. Diabetes Care 2006;29:2688–2693.

20. Rafnsson SB, Deary IJ, Fowkes FG. Peripheral arterial disease and cognitive function. Vasc Med 2009;14:51–61.

21. Hanon O, Haulon S, Lenoir H, et al. Relationship between arterial stiffness and cognitive function in elderly subjects with complaints of memory loss. Stroke 2005;36:2193–2197.

22. Xu G, Zhou Z, Zhu W, Fan X, Liu X. Plasma C-reactive protein is related to cognitive deterioration and dementia in patients with mild cognitive impairment. J Neurol Sci 2009;284:77–80.

23. Blaiko I, Grubeck-Loebenstein B. Role of the immune system in the pathogenesis,
for advanced glycation end products in mild cognitive impairment. J Neural Transm 2008;115:1047–1050
29. Koyama H, Shoji T, Yokoyama H, et al. Plasma level of endogenous secretory RAGE is associated with components of the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:2587–2593
30. Santilli F, Bucciarelli L, Noto D, et al. Decreased plasma soluble RAGE in patients with hypercholesterolemia: effects of statins. Free Radic Biol Med 2007;43:1253–1262
31. Lindsey JB, de Lemos JA, Cipollone F, et al. Association between circulating soluble receptor for advanced glycation end products and atherosclerosis: observations from the Dallas Heart Study. Diabetes Care 2009;32:1218–1220
32. Colhoun HM, Betteridge DJ, Durrington P, et al. Total soluble and endogenous secretory receptor for advanced glycation end products as predictive biomarkers of coronary heart disease risk in patients with type 2 diabetes: an analysis from the CARDS trial. Diabetes 2011;60:2379–2385
33. Semba RD, Ferrucci L, Sun K, et al. Advanced glycation end products and circulating receptors predict cardiovascular disease mortality in older community-dwelling women. Aging Clin Exp Res 2009;21:182–190
34. McNair ED, Wells CR, Mabood Qureshi A, et al. Modulation of high sensitivity C-reactive protein by soluble receptor for advanced glycation end products. Mol Cell Biochem 2010;341:135–138
35. An XF, Zhao Y, Yu JY, Liu JS, Gu WJ, Gao F. Plasma sRAGE is independently associated with high sensitivity C-reactive protein in type 2 diabetes without coronary artery disease. Diabetes Res Clin Pract 2010;87:e19–e22