Letters

Endogenous Secretory Receptor for Advanced Glycation End Product Levels Are Inversely Associated With HbA$_{1c}$ in Type 2 Diabetic Patients

Advanced glycation end products (AGEs) and their receptor (RAGE) system play an important role in the development of diabetic vascular complications (1,2). Recently, an endogenous secretory RAGE (esRAGE) has been identified as a novel splice variant, which lacks the transmembrane domain and is secreted in human sera. Interestingly, it was reported that esRAGE binds AGE ligands and neutralizes AGE actions (3). It is well known that type 2 diabetes is the most prevalent and serious metabolic disease affecting people all over the world and that vascular complications are clinically often observed in type 2 diabetic patients. However, very little information has been obtained about circulating esRAGE levels in type 2 diabetic subjects. To our knowledge, this is the first report examining circulating esRAGE levels in type 2 diabetic patients.

Subjects were selected from outpatients at the Diabetes Clinic of Osaka University Hospital as follows. All type 2 diabetic patients who visited the hospital from June to July 2005 were asked to participate in the study. The determination of type 2 diabetes was based on American Diabetes Association criteria. Those who were suffering from severe renal dysfunction (serum creatinine >2.0 mg/dl), hepatic disease, infection, connective tissue disease, or malignancy were excluded. After all, a total of 147 Japanese type 2 diabetic patients (50 men and 97 women, aged 63.6 ± 9.9 years [mean ± SD], and duration of diabetes 15.1 ± 9.5 years) met the criteria and attended the study. Eleven patients were treated with diet alone, 100 with oral hypoglycemic agents, and 42 with insulin. The study was approved by the Ethical Committee for Human Studies at Osaka University Graduate School of Medicine, and written informed consent was obtained from each subject.

We measured circulating esRAGE levels in serum using the B-Bridge es-RAGE ELISA kit (B-Bridge International, Sunnyvale, CA). The mean ± SD value of esRAGE was 0.394 ± 0.17 ng/ml. BMI (23.9 ± 3.5 kg/m^2), systolic and diastolic blood pressure (132 ± 18 and 74 ± 11 mmHg, respectively), smoking (23.1%), HbA$_{1c}$ (A1C) (7.3 ± 1.3%), total cholesterol (4.84 ± 0.91 mmol/l), triglycerides (1.37 ± 0.82 mmol/l), LDL cholesterol (2.87 ± 0.47 mmol/l), and HDL cholesterol (1.48 ± 0.54 mmol/l) were also evaluated.

Pearson's univariate regression analyses showed that serum esRAGE levels were inversely correlated with A1C ($r = -0.250$, $P = 0.0021$) and total cholesterol ($r = -0.180$, $P = 0.0316$) but positively correlated with HDL cholesterol ($r = 0.237$, $P = 0.0049$). There was no statistically significant association between esRAGE and the other variables. Furthermore, a stepwise multivariate regression analyses demonstrated that high A1C ($F = 7.4$), high total cholesterol ($F = 7.8$), and low HDL cholesterol ($F = 14.4$) were shown to be independent risk factors for a low esRAGE value.

These results suggest that circulating esRAGE levels are related with not only glycemic control but also lipid profiles in type 2 diabetic patients.

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References
1. Stern DM, Yan SD, Yan SF, Schmidt AM: Receptor for advanced glycation end-products (RAGE) and the complications of diabetes. *Ageing Res Rev* 1:1–15, 2002
2. Wautier JL, Schmidt AM: Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 95:233–238, 2004
3. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin J, Li H, Yasui K, Takeuchi M, Makita M, Takasawa S, Okamoto H, Watanabe T, Yamamoto H: Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J* 370:1097–1109, 2003

Insulin Signaling, Glucose Metabolism, and the Angiotensin II Signaling System

Studies in Bartter's/Gitelman's syndromes

Taniyama et al. (1) have recently reported that angiotensin II (Ang II) in vitro decreases insulin receptor substrate-1 protein levels via Src, phosphoinositide-dependent kinase-1, and reactive oxygen species–mediated phosphorylation of Ser307. This leads to the targeting of insulin receptor substrate-1 for proteasome-dependent degradation, which then impairs insulin signaling. These findings provide a rationale for understanding the molecular basis of the positive effect of Ang II type 1 receptor antagonists on insulin resistance.

The relationship between Ang II and insulin signaling shown in vitro leads us to assess whether this is operative also in vivo in humans. We analyzed a cohort of patients with Bartter's/Gitelman's syndrome (BS/GS), which attract much attention for persistent normo-/hypotension despite biochemical and hormonal abnormalities typical of hypertension. BS/GS, caused by gene defects in specific kidney transporters and ion channels, presents hypokalemia, sodium depletion, activation of the renin-angiotensin-aldosterone system, and increased levels of Ang II, yet normo-/hypotension, reduced peripheral resistance, and hyporesponsiveness to pressors (2,3). BS/GS is a good human model to explore the mechanisms responsible for Ang II signaling (2,4). In BS/GS specifically, the short-term Ang II signaling is blunted (increased regulator of G-protein signaling-2 [5], reduced Gαq expression [6,7], and reduced related downstream cellular events [6,8,9]), while the NO system is upregulated (2,10–12). The long-term signaling of Ang II, which modulates the cell redox state to promote cardiovascular remodeling and atherosclerosis, is also altered in BS/GS (13,14). In addition, the RhoA/Rho kinase (ROK) pathway, which is activated by Ang II and shown to affect the Akt–phosphatidylinositol 3-kinase

pathway (15), which, in turn, is involved in glucose transport and metabolism (16), is downregulated in BS/GS (17,18). Thus BS/GS's molecular and biochemical characteristics make it an attractive model to explore whether high Ang II is indeed affecting glucose homeostasis also in vivo in humans.

Six patients with BS/GS (1 with BS, 5 with GS) and 10 normotensive healthy subjects underwent oral glucose tolerance tests to determine not only the glucose tolerance but also, using the oral glucose insulin sensitivity index (19), the glucose clearance as a function of insulin concentration.

All patients showed a normal oral glucose tolerance test at baseline and at 120 min (5.0 ± 0.5 vs. 5.30 ± 0.8 and 6.41 ± 1.9 vs. 5.06 ± 1.2, respectively). Insulin at baseline was significantly reduced compared with control subjects (22.5 ± 9.8 vs. 57.0 ± 26.3, $P = 0.008$), while it was not different at 120 min (198.3 ± 136.0 vs. 190.4 ± 142.7). Oral glucose insulin sensitivity was markedly higher in BS/GS (694.6 ± 103.6 vs. 446.5 ± 48.04 ml $\cdot$ min$^{-1} \cdot$ m^{-2}, $P = 0.00001$).

These results point toward a reduced insulin resistance in BS/GS, therefore not only confirming the blunted nature of Ang II signaling in BS/GS but also supporting in vivo in humans the link between insulin signaling, glucose metabolism, and Ang II signaling demonstrated in vitro (1).

The possible involvement of the RhoA/ROK pathway in glucose metabolism is one more piece of indirect evidence. Ang II, via stimulation of RhoA/ROK activity, inhibits insulin signaling through the inhibition of phosphatidylinositol 3-kinase and its downstream Akt pathway (16), induction of oxidative stress, decreased NO production, increased myosin light-chain activation, vasoconstriction, and reduced glucose transport (16). On the contrary, RhoA/ROK inhibition activates the Akt pathway leading to cardiovascular protection via activation of endothelial NO synthase (15,20). BS/GS shows an upregulation of the NO system (10–12) and downregulation of RhoA/ROK activity (17,18), which may induce Akt pathway supported by BS/GS increased expression of hemeoxygenase-1 (13), which is under Akt control (21).

Finally, the increased expression of p22^{phox} mRNA and oxidative stress as well as the increased expression of p66^{shc} we have shown in type 2 diabetic subjects (22,23) are opposed to the findings in

BS/GS (13), in which preliminary results also show reduced p66^{shc} expression (E.P., L.A.C., personal observation).

In conclusion, our data in BS/GS represent the first direct confirmation in humans of the Ang II/glucose metabolism relationship, thereby supporting the positive effect of blocking the renin-angiotensin-aldosterone system not only for blood pressure control but also glucose tolerance, diabetes, and atherogenesis.

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References
1. Taniyama Y, Hitomi H, Shah A, Alexander RW, Griendling KK: Mechanisms of reactive oxygen species-dependent downregulation of insulin receptor substrate-1 by angiotensin II. *Arterioscler Thromb Vasc Biol* 25:1142–1147, 2005
2. Calò LA, Pessina AC, Semplicini A: Angiotensin II signaling in the Bartter's and Gitelman's syndromes, a negative human model of hypertension. *High Blood Press Cardiovasc Prev* 12:17–26, 2005
3. Naesens M, Steels P, Verberckmoes R, Vanrenterghem Y, Kuypers D: Bartter's and Gitelman's syndromes: from gene to clinic (Review). *Nephron Physiol* 96:65–78, 2004
4. Calò LA: A mirror instead of a looking glass to understand vascular tone control in humans: the utility of studies in Bartter's/Gitelmen's syndromes. *Kidney Int.* In press
5. Calò LA, Pagnin E, Davis PA, Sartori M, Ceolotto G, Pessina AC, Semplicini A: Increased expression of regulator of G protein signaling-2 (RGS-2) in Bartter's/Gitelman's sydrome: a role in the control of vascular tone and implication for hypertension. *J Clin Endocrinol Metab* 89:4153–4157, 2004
6. Calò L, Ceolotto G, Milani M, Pagnin E, van den Heuvel LP, Sartori M, Davis PA, Costa R, Semplicini A: Abnormalities of Gq-mediated cell signaling in Bartter and Gitelman syndromes. *Kidney Int* 60:882–889, 2001
7. Calò L, Davis PA, Semplicini A: Reduced content of alpha subunit of Gq protein in monocytes of Bartter and Gitelman syndromes: relationship with vascular hyporeactivity. *Kidney Int* 61:353–354, 2002
8. Di Virgilio F, Calò L, Cantaro S, Favaro S, Piccoli A, Borsatti A: Resting and stimulated cytosolic free calcium levels in neutrophils from patients with Bartter's syndrome. *Clin Sci* 72:483–488, 1987
9. Calò L, D'Angelo A, Cantaro S, Rizzolo M, Favaro S, Antonello A, Borsatti A: Intracellular calcium signalling and vascular reactivity in Bartter's syndrome. *Nephron* 72:570–573, 1996
10. Calò L, Davis PA, Milani M, Cantaro S, Antonello A, Favaro S, D'Angelo A: Increased endothelial nitric oxide synthase mRNA level in Bartter's and Gitelman's syndrome: relationship to vascular reactivity. *Clin Nephrol* 51:12–17, 1999
11. Calò L, D'Angelo A, Cantaro S, Bordin MC, Favaro S, Antonello A, Borsatti A: Increased urinary NO$_2^-$/NO$_3^-$ and cyclic GMP levels in patients with Bartter's syndrome: relationship to vascular reactivity. *Am J Kidney Dis* 27:874–879, 1996
12. Calò L, Cantaro S, Calabro A, Piarulli F, Rizzolo M, Favaro S, Antonello A, Crepaldi G, Borsatti A: Endothelium-derived vasoactive substances in Bartter's syndrome. *Angiology* 46:905–913, 1995
13. Calò LA, Pagnin E, Davis PA, Sartori M, Semplicini A: Oxidative stress related factors in Bartter's and Gitelman's syndromes: relevance for angiotensin II signalling. *Nephrol Dial Transplant* 18:1518–1525, 2003
14. Calò L, Sartore G, Bassi A, Basso C, Bertocco S, Marin R, Zambon S, Cantaro S, D'Angelo A, Davis PA, Manzato E, Crepaldi G: Reduced susceptibility of low density lipoprotein to oxidation in patients with overproduction of nitric oxide (Bartter's and Gitelman's syndrome). *J Hypertens* 16:1001–1008, 1998
15. Wolfrum S, Dendorfer A, Rikitake Y, Stalker TJ, Gong Y, Scalia R, Dominiak P, Liao PK: Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3 kinase/protein kinase Akt and cardiovascular protection. *Arterioscler Thromb Vasc Biol* 24:1842–1847, 2004
16. Sowers JR: Insulin resistance and hypertension. *Am J Physiol Heart Circ Physiol* 286:H1597–H1602, 2004
17. Pagnin E, Davis PA, Sartori M, Semplicini A, Pessina AC, Calò LA: Rho kinase and PAI-1 in Bartter's/Gitelman's syndromes: relationship to angiotensin II signaling. *J Hypertens* 22:1963–1969, 2004
18. Pagnin E, Semplicini A, Sartori M, Pessina AC, and Calò LA: Reduced mRNA and protein content of Rho guanine nucleotide exchange factor (RhoGEF) in Bartter's and Gitelman's syndromes: relevance for the pathophysiology of hypertension. *Am J Hypertens* 18:1200–1205, 2005
19. Mari A, Pacini G, Murphy E, Ludvik B,

Nolan JJ: A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care* 24:539–548, 2001

20. Mita S, Kobayashi N, Yoshida K, Nakano S, Matsuoka H: Cardioprotective mechanisms of Rho-kinase inhibition associated with eNOS and oxidative stress-LOX-1 pathway in Dahl salt-sensitive hypertensive rats. *J Hypertens* 23:87–96, 2005

21. Martin D, Rojo AI, Salinas M, Diaz R, Gallardo G, Alam J, De Galarreta CM, Cuadrado A: Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. *J Biol Chem* 279:8919–8929, 2004

22. Avogaro A, Pagnin E, Calo L: Monocyte NADPH oxidase subunit p22(phox) and inducible hemeoxygenase-1 gene expressions are increased in type II diabetic patients: relationship with oxidative stress. *J Clin Endocrinol Metab* 88:1753–1759, 2003

23. Pagnin E, Fadini G, de Toni R, Tiengo A, Calo L, Avogaro A: Diabetes induces p66shc gene expression in human peripheral blood mononuclear cells: relationship to oxidative stress. *J Clin Endocrinol Metab* 90:1130–1136, 2005

Exenatide (Exendin-4)–Induced Pancreatitis

A case report

Exenatide is a 39–amino acid peptide approved for the adjunctive treatment of type 2 diabetes. It is an incretin mimetic agent that is consistent in activity with the actions of glucagon-like peptide 1. Proposed mechanisms of action include enhanced glucose-dependent insulin secretion from pancreatic β-cells, restoration of first-phase insulin response, suppression of glucagon secretion, and delay of gastric emptying. Kendall et al. (1) found no evidence of cardiovascular, pulmonary, hepatic, or renal toxicities with exenatide. Nausea (39–48%) and hypoglycemia (19–27%) were the most common side effects reported.

A 69-year-old man with type 2 diabetes of 15 years' duration presented for follow-up. He had known diabetic neuropathy and retinopathy. His medical history was remarkable for coronary artery disease, gastroesophageal reflux disease, rheumatoid arthritis, and colonic polyposis. He was taking metformin at 500 mg p.o., a.c., b.i.d.; pioglitazone at 30 mg p.o. daily; NPH insulin at 45 units s.q., a.c., in the morning, and 20 units s.q., a.c., in the evening; insulin aspart on a sliding scale; metoprolol at 50 mg p.o. daily; gabapentin at 1,200 mg p.o. daily; lovastatin at 40 mg p.o. daily; irbesartan at 150 mg p.o. at bedtime; clopidogrel at 75 mg p.o. daily; infliximab at 3 mg/kg i.v. every 8 weeks; ezetimibe 10 mg p.o. daily; and esomeprazole at 40 mg p.o. daily. Remarkable findings on examination were exogenous obesity, bilateral retinal dot hemorrhages, trace pitting bipedal edema, hyperpigmentation of the legs, and a symmetric distal stocking polyneuropathy. The patient was 6 ft tall and weighed 268 lb. HbA$_{1c}$ level was 10.5%.

Treatment options were discussed, and exenatide at 5 mg s.q. b.i.d. was initiated. The pioglitazone and the metformin were discontinued. Within 24 h of initiating the exenatide, the patient developed a midepigastric abdominal pain that radiated through to the back. As he continued with the exenatide therapy the pain intensified. There was no fever or chills. He denied alcohol use or exposure to new medication. There was no previous history of pancreatitis or gallstones.

The patient presented to the emergency room on the 5th day of therapy. He was noted to have a glucose level of 309 mg/dl, creatinine of 1.0 mg/dl, and CO_2 of 27, and ketones were negative. Aspartate aminotransferase was 25 IU/l and alanine aminotransferase 25 IU/l. Serum triglycerides were 150 mg/dl, serum calcium was 8.6 mg/dl, white blood cell count was 11,000, and hemoglobin was 13.8 g/l. Serum amylase was 384 IU/l and serum lipase 346 IU/l. Computed axial tomography scan of the abdomen revealed no evidence of cholelithiasis. The presumptive diagnosis of acute pancreatitis was made. Intravenous fluids along with intravenous pantoprazole were started. He was made NPO (nothing to eat), and a gastroenterologic consultation was obtained. The NPH and the exenatide were withheld. A weight-based sliding scale of insulin was started using aspart.

On subsequent days the lipase was 106, 27, and 17 IU/l. The abdominal pain resolved by day 3. Clear fluids were started, and the diet was advanced without difficulty. The patient was discharged home without sequelae.

We report a case of acute pancreatitis in which exenatide appears to be the etiologic agent. A review of the literature failed to reveal any previously reported cases of exenatide-induced acute pancreatitis. An occult etiology for the pancreatitis cannot be completely discounted. Pancreatitis has been reported with mevacor, infliximab, and gabapentin, but their protracted use without change in dose mitigates their being the etiologic agent. The temporal relation of the symptoms to the onset and cessation of therapy along with the normalization of laboratory parameters on drug withdrawal implicates exenatide as the cause. Caution should be exercised when prescribing exenatide with agents known to cause pancreatitis and in patients at high risk.

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References
1. Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD: Effects of exenatide (Exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. *Diabetes Care* 28:1083–1091, 2005

The Use of Insulin Glargine With Gestational Diabetes Mellitus

We agree with the recent letter by Woolderink et al. (1) that insulin glargine use during pregnancy may be appropriate. In contrast to that letter, which described the use of insulin glargine in pregnant women with type 1 diabetes, we detail the use of insulin glargine in four patients with gestational diabetes mellitus (GDM). Target blood glucose levels set by the American College of Obstetricians and Gynecologists for women with GDM include fasting glucose ≤95 mg/dl and 1-h postprandial glucose ≤130–140 mg/dl or 2-h postprandial glucose ≤120 mg/dl (2). These criteria are used by the Maternal-Fetal Medicine