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Positive association between serum levels of advanced glycation end products and the soluble form of receptor for advanced glycation end products in nondiabetic subjects

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Abstract

The advanced glycation end products (AGEs)-receptor for AGE (RAGE) axis is implicated in diabetic vascular complications. Administration of soluble form of RAGE (sRAGE) to mice has been shown to block the AGE-elicited tissue damage by acting as a decoy. These observations suggest that endogenous sRAGE may capture and eliminate circulating AGEs and decrease its serum levels. However, because AGEs up-regulate tissue RAGE expression and endogenous sRAGE could be generated from the cleavage of cell surface RAGE, sRAGE may be positively, rather than inversely, associated with circulating AGEs by reflecting tissue RAGE expression. In this study, we investigated the association of sRAGE with serum levels of AGEs in humans. Data for fasting serum sRAGE and AGE levels of 184 nondiabetic subjects were obtained from a general population in Japan. We also measured body mass index (BMI), waist circumference, blood pressure, and blood biochemistries in this population. Uni- and multivariate analyses were applied for the determinants of serum sRAGE levels. The average sRAGE levels were 0.40 ± 0.17 ng/mL in males and 0.43 ± 0.14 ng/mL in females, respectively. In the univariate analysis, BMI ($P < .05$, inversely), waist circumference ($P < .05$, inversely), AGEs ($P < .05$), and alcohol intake ($P < .05$, inversely) were significantly associated with sRAGE levels. After performing multivariate analyses, BMI ($P < .05$, inversely) and AGEs ($P < .05$) still remained significant independently. The present study is the first demonstration that serum sRAGE levels were positively associated with circulating AGEs in the nondiabetic general population. Endogenous sRAGE levels are elevated in parallel with serum AGE levels.

1. Introduction

Reducing sugars can react non-enzymatically with amino groups of protein to form Amadori products. These early glycation products undergo further complex reaction such as rearrangement, dehydration, and condensation to become irreversibly cross-linked, heterogeneous fluorescent derivatives, termed *advanced glycation end products* (AGEs) [1-4]. The formation and accumulation of AGEs have been known to progress at an accelerated rate in diabetes. Recent understanding of this process confirmed that the AGE-receptor for AGE (RAGE) axis was implicated in the pathogenesis of diabetic vascular complications [5-9]. Furthermore, recently, administration of a recombinant soluble form of RAGE (sRAGE) consisting of the extracellular ligand-binding domain has been shown not only to suppress the development of atherosclerosis, but also to stabilize established atherosclerosis in diabetic apolipoprotein E–null mice [10,11]. These observations suggest that exogenously administered sRAGE may capture and eliminate circulating AGEs, thus protecting against the AGE-elicited tissue damage by acting as a decoy.

Based on these observations, endogenous sRAGE could be one of the determinants of circulating AGE levels, and therefore, inversely correlated with serum AGEs. However, because AGEs up-regulate RAGE expression in various tissues [5,7] and that endogenous sRAGE could be generated from the cleavage of cell surface RAGE by the

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Table 1
Characteristics of 184 subjects

	Men (n = 62)	Women (n = 122)	Total (N = 184)
Age (y)	66.7 ± 9.1	65.0 ± 9.5	65.6 ± 9.4
BMI (kg/m^2)	23.2 ± 3.7	23.5 ± 3.3	23.4 ± 3.4
Waist circumference (cm)	87.8 ± 6.5	89.1 ± 7.8	88.7 ± 7.5
Systolic BP (mm Hg)	131.9 ± 15.4	139.4 ± 19.9	136.8 ± 18.8
Diastolic BP (mm Hg)	82.6 ± 11.5	81.4 ± 12.6	81.8 ± 12.2
AGEs (U/mL)	4.1 ± 0.7	4.1 ± 0.9	4.1 ± 0.8
sRAGE (ng/mL)	0.40 ± 0.17	0.43 ± 0.14	0.42 ± 0.15
Total cholesterol (mg/dL)	191.4 ± 34.3	207.6 ± 34.1	202.0 ± 34.9
LDL-C (mg/dL)	106.7 ± 27.7	123.7 ± 31.4	117.9 ± 31.2
HDL-C (mg/dL)	58.6 ± 15.8	60.4 ± 13.9	59.8 ± 14.6
Triglycerides (mg/dL)[a]	93.7 ± 16.6	94.8 ± 40.2	100.6 ± 36.5
Glucose (mg/dL)[a]	99.6 ± 11.7	91.5 ± 11.2	94.7 ± 11.3
HbA_{1c} (%)	5.13 ± 0.32	5.18 ± 0.31	5.16 ± 0.32
Insulin (μU/mL)[a]	4.3 ± 2.5	4.0 ± 1.9	4.2 ± 2.1
HsCRP (mg/dL)[a]	0.047 ± 0.012	0.033 ± 0.011	0.037 ± 0.014
Ccr (mL/min)	81.7 ± 27.8	80.7 ± 22.9	80.0 ± 24.7
Uric acid (mg/dL)	5.7 ± 1.4	4.6 ± 1.1	5.0 ± 1.3
Total protein (g/dL)	7.2 ± 0.4	7.4 ± 0.4	7.3 ± 0.4
Frequency (%)			
HT medication	25.8	30.3	28.8
HL medication	7.8	9.0	8.6
Alcohol intake	73.0	14.8	34.6
Current smoking	23.4	0.8	8.6

Values are expressed as mean ± SD or percentage, unless indicated otherwise. LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Ccr, creatinine clearance (calculated by the Cockcroft-Gault equation); HT, hypertension; HL, hyperlipidemia.

[a] Log-transformed values were used for the calculation of means and SD and exponentiated geometric means are presented.

actions of matrix metalloproteinases [12,13], sRAGE may be positively, rather than inversely, associated with circulating AGEs and may reflect tissue RAGE expression. Therefore, in this study, we measured serum levels of sRAGE and AGEs simultaneously and investigated the relationship between these 2 parameters in humans.

2. Methods

2.1. Subjects

In 2004, in a fishing community in southwestern Japan (Uku town), a total of 204 people received a health examination. This town is an isolated island near Fukue city, located in Nagasaki prefecture, and the total population is about 3700. We have been performing an epidemiologic study every year for 4 years. The subjects' demographic backgrounds of this year were almost same to those of the previous years. Of these, 8 subjects rejected blood test. We excluded 12 diabetic subjects (hemoglobin A_{1c} [HbA_{1c}] levels of >6.5% and/or fasting plasma glucose of >126 mg/dL). Finally, complete data set for 184 nondiabetic subjects (62 males and 122 females) was available in this study.

2.2. Data collection

The medical history, smoking, and alcohol intake were ascertained by a questionnaire. Smoking and alcohol intake were classified as current habitual use or not. Height and weight were measured, and body mass index (BMI; weight in kilograms divided by the square of height in meters) was calculated as an index of obesity. Blood pressure (BP) was measured in the sitting position (first) and supine position (second) at 3-minute interval using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 minutes before BP measurement. The supine BP with the fifth-phase diastolic pressure was used for analysis.

Blood was drawn from the antecubital vein in the morning after 12-hour fast for determinations of lipids (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), fasting plasma glucose, glycosylated HbA_{1c}, insulin, creatinine, uric acid, total protein, high-sensitivity C-reactive protein (hsCRP), AGEs, and sRAGE. Serum AGE levels were measured with an enzyme-linked immunosorbent assay (ELISA) as described previously [14]. In this study, 1 U corresponds to 2 μg of glyceraldehyde-derived AGE–bovine serum albumin standard as described previously [14]. The sRAGE levels were determined with a commercially available ELISA kit (R&D systems, Minneapolis, MN). Interassay (n = 40) and intra-assay (n = 20) coefficient of variations of the sRAGE ELISA were 7.7% and 5.7%, respectively. Other chemistries (enzymatic assay method) were measured at a commercially available laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan). Creatinine clearance was estimated with the Cockcroft-Gault equation [15]. The mayor and the welfare section of Uku town approved this study. The ethical committee of the Kurume University also approved this study. All participants gave informed consent.

2.3. Statistical methods

Because of skewed distributions, the natural logarithmic transformations were performed for triglycerides, glucose, insulin, and hsCRP. Mean values with upper and lower 95% confidence intervals (CI) were exponentiated and presented

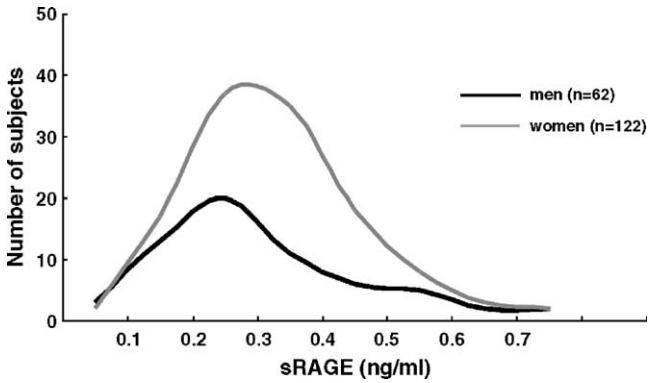

Fig. 1. Distributions of serum sRAGE levels in men and women.

Table 2
Association between sRAGE and other parameters

	β	SE	P
Age	−.062	0.001	.360
Sex	.097	0.023	.298
BMI	−.177	0.003	.023
Waist circumference	−.175	0.001	.028
Systolic BP	−.017	0.001	.682
Diastolic BP	.010	0.001	.918
AGEs	.152	0.013	.039
Total cholesterol	.050	0.001	.597
LDL-C	.108	0.001	.234
HDL-C	.078	0.001	.165
Triglycerides[a]	−.034	0.025	.924
Glucose[a]	−.138	0.015	.232
HbA_{1c}	.015	0.035	.842
Insulin[a]	−.037	0.014	.617
HsCRP[a]	−.041	0.010	.581
Ccr	−.135	0.001	.067
Uric acid	−.027	0.009	.720
Total protein	.047	0.028	.601
HT medication	−.107	0.024	.149
HL medication	.121	0.039	.101
Alcohol intake	−.155	0.023	.033
Current smoking	.029	0.039	.710

β indicates standardized regression coefficients. Men = 0, women = 1; medication: no = 0, yes = 1.

[a] Log-transformed values were used.

as geometric mean ± SD, where the SD was approximated as the difference of the exponentiated CI divided by 3.92, which is the number of SD in a 95% CI where data are normally distributed. Results are presented as mean ± SD. The medications for hypertension and hyperlipidemia were coded as dummy variables. Univariate analysis was performed for determinants of serum sRAGE levels. Multiple linear regression analysis was performed to determine independent determinants of sRAGE levels. Statistical significance was defined as $P < .05$. All statistical analyses were performed with the use of the SPSS system (SPSS, Chicago, IL).

3. Results

Backgrounds of the subjects are presented in Table 1. Mean BMI in both sexes was 23.5 kg/m^2. Only 15 subjects (8.2%) met the new criteria of the metabolic syndrome for Japanese proposed by the International Diabetes Federation [16]. The levels of sRAGE did not differ between men and women. Fig. 1 shows distributions of sRAGE levels in men and women, which shows normal distributions in both sexes. Table 2 shows results of univariate analysis for determinants of serum sRAGE levels. Parameters statistically and significantly related to sRAGE levels were AGEs ($P < .05$), BMI ($P < .05$, inversely), waist circumference ($P < .05$, inversely), and alcohol intake ($P < .05$, inversely). Because these significant parameters could be closely correlated each other, multiple linear regression analysis was performed. Finally, BMI ($P < .05$, inversely) and AGE levels ($P < .05$) remained significant (Table 3) and were independently related to serum sRAGE levels ($R^2 = 0.145$). The metabolic syndrome was not associated with sRAGE levels ($P = .698$). Alcohol intake or waist circumference did not affect the levels of sRAGEs by multivariate analysis. Even after including 12 diabetic patients, BMI ($P < .05$, inversely) and AGE levels ($P < .05$) still remained significant.

4. Discussion

In the present study, we demonstrated for the first time that serum sRAGE levels in nondiabetic subjects were positively, rather than inversely, associated with AGE levels. Age-, sex- and BMI-adjusted AGE levels were significantly increased, but not decreased, in proportion to the increasing levels of sRAGE (data not shown). These findings suggest that endogenous sRAGE could not efficiently capture and eliminate circulating AGEs in vivo. Because AGEs are positive regulators of cell expression of RAGE [17-19], our present observations suggest that circulating sRAGE levels may reflect tissue RAGE expression and may be elevated in parallel with serum AGE levels as a counter-system against the AGE-elicited tissue damage, even in a nondiabetic general population without apparent cardiovascular disease or renal disease.

The concept that endogenous sRAGE may reflect tissue RAGE expression is supported by several observations. (1) The receptor for AGE is a cell surface receptor that belongs to the immunoglobulin superfamily such as intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) [5]. Levels of the soluble form of ICAM-1 and VCAM-1 are elevated in various disorders by reflecting up-regulation of these molecules in endothelial cells (ECs) [20]. (2) Angiotensin II increases RAGE messenger RNA levels in ECs and subsequently stimulates sRAGE formation. Treatments with telmisartan, an angiotensin II type 1 receptor blocker, not only inhibits the angiotensin II–elicited sRAGE generation by ECs, but also decreases serum levels of sRAGE in patients with essential hypertension [21]. (3) Vitreous levels of sRAGE are increased in proliferative retinal diseases by reflecting enhanced expression of RAGE in epiretinal membranes of the eyes [22].

4.1. Subject selection

We limited our analysis to nondiabetic subjects without apparent cardiovascular disease or renal disease for some

Table 3
Multiple stepwise regression analysis to determine sRAGE levels

Parameters	β	SE	P
BMI	−.167	0.003	.019
AGEs	.157	0.013	.032
Alcohol intake	−.143	0.023	.053
Waist circumference	−.119	0.024	.113

$R^2 = 0.145$.

reasons. First, serum AGEs levels are reported to be elevated in diabetic or nondiabetic subjects with coronary artery disease or renal dysfunction, which could confound the relationship between serum AGE and sRAGE levels [23-25]. Second, among 204 subjects enrolled in this study, only 12 patients had diabetes. Although about 30% of our subjects received medication for hypertension (Table 1), it is unlikely that antihypertensive drugs could confound the present results because univariate analysis revealed no significant correlation between the medication for hypertension and serum sRAGE levels (Table 2).

4.2. Serum levels of sRAGE

As far as we know, there are 2 articles to report sRAGE levels in nondiabetic general population without apparent cardiovascular disease or renal disease [26,27]. Koyama et al [26] reported that sRAGE levels in nondiabetic Japanese were inversely associated with BMI. These observations were consistent with the present finding that sRAGE levels were inversely correlated with BMI. However, our results were not consistent with another observation by Geroldi et al [27]. They showed that sRAGE was decreased in patients with hypertension compared with normotensive controls (1.21 vs 1.36 ng/mL, $P < .01$) and that pulse pressure was the only determinant of sRAGE levels in white Italians. Their values were much higher than those in our study, and BMI had no relationship with sRAGE levels in their study. We do not know the reasons for the different results and different values of sRAGE levels between our study and theirs. The difference of subject population (age) and ethnicity could account for the discrepancies.

Recently, Falcone et al [28] reported that low levels of sRAGE were independently associated with the presence of coronary artery disease in nondiabetic men, thus suggesting that decreased levels of circulating sRAGE may represent a marker and/or mediator of vulnerability to atherosclerosis. Because we measured here serum levels of sRAGE in nondiabetic subjects without apparent cardiovascular disease or renal disease, the association of sRAGE with cardiovascular disease is not known from our study.

Hemoglobin A_{1c} is one of the early glycation products. Therefore, we studied here the correlation between HbA_{1c} and sRAGE in our subjects. In this study, we did not find any association of HbA_{1c} with serum sRAGE levels (Table 2). This lack of association may be caused by a different turnover in AGEs and HbA_{1c} [22].

5. Limitation

First, because endogenous sRAGE could be generated from the cleavage of cell surface RAGE proteins or novel splice variants of RAGE [12,29-31], the assay used here does not distinguish the types of endogenous sRAGE. Therefore, although we have already shown that the splice variant-derived sRAGE was capable of binding AGEs [29], we do not know whether all the material that we measured here was active and capable of binding AGEs. Second, it would be interesting to analyze the relationship between sRAGE and other known biomarkers of cardiovascular disease such as soluble forms of ICAM-1 and VCAM-1, although sRAGE levels were not correlated with hsCRP in our subjects. Third, our study was limited to nondiabetic subjects without apparent cardiovascular disease or renal disease. It is interesting to study a large number of subjects including nondiabetic as well as diabetic subjects with or without cardiovascular disease or renal disease. Fourth, our study was a cross-sectional one and, therefore, does not elucidate the causal relationships between AGEs and sRAGE levels. Eventually, a longitudinal study is needed to clarify the causal relationship between sRAGE levels and tissue damage or cardiovascular events. We would continue the study and collect the data over several years for more detailed evaluation.

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References

[1] Vlassara H, Bucala R, Striker L. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. Lab Invest 1994;70:138-50.
[2] Brownlee M. Advanced protein glycosylation in diabetes and aging. Annu Rev Med 1995;46:223-34.
[3] Dyer DG, Blackledge JA, Thorpe SR, Baynes JW. Formation of pentosidine during nonenzymatic browning of proteins by glucose. J Biol Chem 1991;18:11654-60.
[4] Grandhee S, Monnier VM. Mechanism of formation of the Maillard protein cross-link pentosidine. J Biol Chem 1991;18:11649-53.
[5] Schmidt AM, Stern D. Atherosclerosis and diabetes: the RAGE connection. Curr Atheroscler Rep 2000;5:430-6.
[6] Lander HM, Tauras JM, Ogiste JS, et al. Activation of the receptor for advanced glycation end products triggers a p21ras-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. J Biol Chem 1997;272:17810-4.
[7] Yamagishi S, Imaizumi T. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. Curr Pharm Des 2005;11:2279-99.
[8] Takeuchi M, Kikuchi S, Sasaki N, et al. Involvement of advanced glycation end-products (AGEs) in Alzheimer's disease. Curr Alzheimer Res 2004;1:39-46.
[9] Abe R, Shimizu T, Sugawara H, et al. Regulation of human melanoma growth and metastasis by AGE–AGE receptor interactions. J Invest Dermatol 2004;122:461-7.

[10] Park L, Raman KG, Lee KJ, et al. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation end products. Nat Med 1998;4:1025-31.
[11] Bucciarelli L, Wendt T, Qu W, et al. RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E–null mice. Circulation 2002;106:2827-35.
[12] Hudson BI, Harja E, Moser B, Schmidt AM. Soluble levels of receptor for advanced glycation endproducts (sRAGE) and coronary artery disease. The next C-reactive protein? Arterioscler Thromb Vasc Biol 2005;25:879-82.
[13] Hanford LE, Enghild JJ, Valnickova Z, et al. Purification and characterization of mouse soluble receptor for advanced glycation end products (sRAGE). J Biol Chem 2004;279:50019-24.
[14] Takeuchi M, Makita Z, Bucala R, et al. Immunological evidence that non-carboxymethyllysine advanced glycation end-products produced from short chain sugars and dicarbonyl compounds in vivo. Mol Med 2000;6:114-25.
[15] McAlister FA, Ezekowitz J, Tonelli M, Armstrong PW. Renal insufficiency and heart failure. Prognostic and therapeutic implications from a prospective cohort study. Circulation 2004;109: 1004-9.
[16] Grundy SM, Cleeman CJ, Daniels SR, et al. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735-52.
[17] Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products. A mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ Res 1999;84:489-97.
[18] Tanaka N, Yonekura H, Yamagishi S, et al. The receptor for advanced glycation end products is induced by the glycation products themselves and tumor necrosis factor–alpha through nuclear factor–kappa B, and by 17beta-estradiol through Sp-1 in human vascular endothelial cells. J Biol Chem 2000;75:25781-90.
[19] Yamagishi S, Amano S, Inagaki Y, et al. Beraprost sodium, a prostaglandin I_2 analogue, protects against advanced glycation end products–induced injury in cultured retinal pericytes. Mol Med 2002;8:546-50.
[20] Ferri C, Desideri G, Baldoncini R, et al. Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. Diabetes 1998;47:66-667.
[21] Nakamura K, Yamagishi S, Nakamura Y, et al. Telmisartan inhibits expression of a receptor for advanced glycation end products (RAGE) in angiotensin-II–exposed endothelial cells and decreases serum levels of soluble RAGE in patients with essential hypertension. Microvasc Res 2005;70:137-41.
[22] Pachydaki SI, Tari SR, Lee SE, et al. Upregulation of RAGE and its ligands in proliferative retinal disease. Exp Eye Res doi:10.1016/j.exer.2005.09.022.
[23] Kilhovd BK, Berg TJ, Birkeland KI, Thorsby P, Hanssen KF. Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease. Diabetes Care 1999; 22:1543-8.
[24] Kanauchi M, Hashimoto T, Tsujimoto N. Advanced glycation end products in nondiabetic patients with coronary artery disease. Diabetes Care 2001;24:1620-3.
[25] Selvaraj N, Bobby Z, Das AK, Ramesh R, Koner BC. An evaluation of level of oxidative stress and protein glycation in nondiabetic undialyzed chronic renal failure patients. Clin Chim Acta 2002;324:45-50.
[26] 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; 25:2587-93.
[27] Geroldi D, Falcone C, Emanuele E, et al. Decreased plasma levels of soluble receptor for advanced glycation end-products in patients with essential hypertension. J Hypertens 2005;23:1725-9.
[28] Falcone C, Emanuele E, D'Angelo A, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arterioscler Thromb Vasc Biol 2005;25: 1032-7.
[29] Yonekura H, Yammoto Y, Sakurai S, et al. Novel splice variants of the receptor for advanced glycation endproducts expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem J 2003;370:1097-109.
[30] Schlueter C, hanke S, Flohr AM, Rogalla P, Bullerdiek J. Tissue specific expression patterns of RAGE receptor and its soluble forms—a result of regulated alternative splicing? Biochim Biophys Acta 2003;1630:1-6.
[31] Park IH, Yeon SI, Youn JH, et al. Expression of a novel secreted splice variant of the receptor for advanced glycation endproducts (RAGE) in human brain astrocytes and peripheral blood mononuclear cells. Mol Immunol 2004;40:1203-11.