Pathobiological Role of Advanced Glycation Endproducts via MAPK Dependent Pathway in the Diabetic Vasculopathy

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Pathobiological Role of Advanced Glycation Endproducts via MAPK Dependent Pathway in the Diabetic Vasculopathy

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Abstract

Pathobiological role of advanced glycation endproducts via MAPK dependent pathway in the diabetic vasculopathy

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Advanced glycation endproducts (AGEs) enhance the immunoinflammatory reaction in diabetic vasculopathy through receptors for AGE (RAGE). Recently, AGEs have been reported to play a role in neointimal formation and increase the rate of in-stent restenosis (ISR) in the diabetic coronary artery disease patients treated with stents. However, the potential roles and pathogenic mechanisms of AGE in vascular smooth muscle cell (VSMC) proliferation and ISR remain unclear. We sought to determine the AGEs related pathobiological mechanism of diabetic vasculopathy. Serum levels of AGEs were analyzed by fluorescent intensity method in the diabetic PCI patients. High AGEs level was

independent risk factors for ISR by multivariate analysis. The RAGE expression in human atheroma was assessed by immunohistochemistry. Rat aortic smooth muscle cell (RAoSMC) culture was done with AGEs stimulation. Western blotting was performed to assess the activation of mitogen-associated protein kinase(MAPK) system in the cultured VSMC. AGEs stimulated VSMC proliferation and were associated with increased phosphorylation of ERK, JUN, and p38 by time and dose dependent manner. The ERKs activation by AGEs was decreased by RNA interference for RAGE, indicating that AGEs activated ERKs via RAGE. AGEs may play a key role in VSMC proliferation and increase ISR in patients with diabetes melitus. Activation of VSMC proliferation by MAPK system and increased formation of ROS may be the possible mechanism of AGEs induced diabetic vasculopathy

Key words: AGEs, MAPK, restenosis, diabetic vasculopathy.

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I. INTRODUCTION

The factors underlying accelerated atherosclerosis in diabetes extend beyond dyslipidemia, hypertension and obesity. Even after correction of these typical risk factors and rigorous control of hyperglycemia, diabetic patients continue to experience increased atherosclerotic vascular disease so called diabetic vasculopathy[1-3]. As a consequence of increased coronary artery diseases (CAD) in diabetic patients, the frequency of percutaneous coronary intervention (PCI) is increased nowadays but, restenosis after PCI remains a significant limitation affecting about over 30% of patients treated with PCI in diabetes[4]. It is assumed to be the result of neointimal formation characterized by migration and proliferation of vascular smooth muscle cells; however, the pathogenic mechanisms leading to the exaggerated restenosis in diabetes are poorly

understood[5].

AGEs, resulting from nonenzymatic glycation and oxidation of proteins or lipids, accumulate continuously on long-lived vessel wall in diabetes and have been linked to the development of diabetic complications[6]. Recent studies have demonstrated that AGEs and its receptor/ligand interaction plays a key role in neointimal formation after vascular injury irrespective of diabetes status and these findings suggest a novel target to minimize neointimal hyperplasia[7-9]. Activation of RAGE results in both removal of irreversibly glycosylated molecules, and activation of cell function, including secretion of various cytokines[10]. AGE stimulate the secretion of tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β) may activate mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB)-dependent pathways and subsequently induce the proinflammatory mediators[11]. However, the role of AGEs in the signaling pathways of vascular smooth muscle cell (VSMC) proliferation remains to be elucidated.

We hypothesized that AGEs may be associated with ISR in diabetic PCI patients and AGE induced increased VSMC proliferation maybe the pathobiologic mechanism. AGE is associated with activation of the MAPK system, an important signaling pathway associated with VSMC proliferation.

Furthermore, we also investigated the association between AGEs and formation of ROS.

II. MATERIALS AND METHODS

1. Seroepidemiologic study and measurement of AGEs

A consecutive series of diabetic patients (n=203), who had symptomatic coronary artery disease treated with stenting and undergone the follow-up angiography were enrolled in this study. All patients were treated with balloon expandable, slotted tube or modular designed bare metal stents. A daily dose of aspirin and clopidogrel was administered for four weeks after the PCI. At the 6 months follow-up angiography, ISR was defined as >50% in stent stenosis measured by QCA analysis.

Blood samples were collected at the time of initial coronary angiography and were transferred to tubes without EDTA. After centrifuge, serum samples were mixed with 480 μL of trichloroacetic acid (0.15 mol/L) in microcentrifuge tubes, and 100 μL of chloroform was added. The serum levels of AGEs were determined by fluorescent intensity method using a fluorescence

spectrophotometer (F-2000 Hitachi Ltd., Tokyo, Japan) at an emission wavelength of 440 nm with an excitation wavelength of 370 nm. The samples were run in triplicate and peak height mode was used for signal measurements. The intra and inter-assay coefficients of variation for AGEs determination were 7.9% and 10.8%, respectively[12]. We tentatively classified these patients using the ROC analysis (Figure 1).

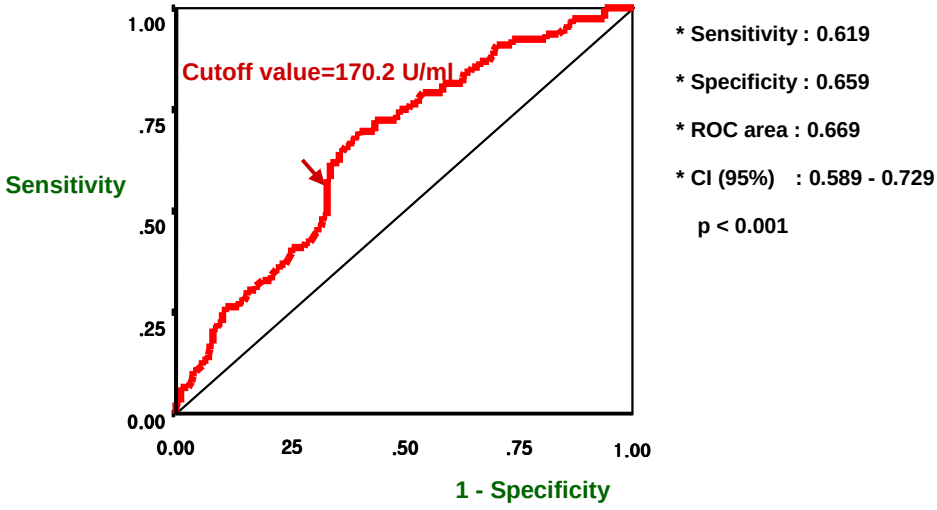


Figure 1. Receiver operating characteristics (ROC) curve of the serum levels of AGEs for the in-stent restenosis (ISR). ROC area was 0.659; 95% CI 0.589 to 0.729; $p < 0.001$. The cutoff point for levels of AGEs was 170.2 U/ml (sensitivity=0.619, specificity=0.659).

Patients with the level of AGEs > 170 U/ml were classified as the high-AGEs group, those with ≤ 170 U/ml as the low-AGEs group. By this classification, the high- and low-AGEs groups consisted of 85 patients (115 lesions) and 118 patients (148 lesions), respectively.

2. Tissue preparation and immunochemistry in human atheroma specimens.

Human carotid endarterectomy specimens (n=9) were obtained from diabetic patients undergone carotid endarterectomy. Each specimen was fixed with 10% buffered formalin and embedded in paraffin. Immunohistochemistry was performed using polyclonal monospecific antibodies to RAGE (Santa Cruz Biotech. CA, USA). Peroxidase-conjugated goat anti-rabbit IgG (Sigma. St.louis. Mo. USA)) was used to visualize the sites of primary antibody binding to the antigen.

3. Isolation and culture of rat aortic smooth muscle cells(RAoSMC) and preparation of AGEs

RAoSMCs were isolated as previously described[13]. The aorta was enzymatically isolated from the thoracic aortas from 6-8 weeks-old Spraque-

Dawley rats. The aorta was transferred into a plastic tube containing 5 ml of the enzyme dissociation mixture and was incubated for 2 hrs at 37℃. The suspension was centrifuged (1500 rpm for 10 min) and the pellet was resuspended in DMEM with 10% fetal bovine serum (FBS). Cells were cultured over several passages (up to 15). RAoSMC were cultured in DMEM supplemented with 10% FCS, 100 IU/ml penicillin, 100μg/ml streptomysin in 75-cm^2 flasks at 37℃ in a humidified atmosphere of 90% air and 10% CO2 (Forma Scientific. FL. USA). RAoSMCs culture with different concentration (1.0, 10, 100, 1000 μg/mL) of AGEs stimulation. AGE–bovine serum albumin (AGE-BSA) was prepared by incubating BSA (WAKO, Tokyo, Japan) in phosphate-buffered saline with 0.7 mol/L glucose for 6 months at 37°C.

4. Measurement of cell proliferation

Cell proliferation was measured by PreMix WST-1 Cell Proliferation Assay System (TAKARA Biomedicals. Tokyo. Japan). This system enables the measurement of cell proliferation with colorimetric assay, and bases on the cleavage of slightly red tetrazolium salt (WST-1) by mitochondrial succinate-tetrazolium reductase in viable cells. As the increase in enzymes activity leads to an increase of the production of formazan dye, the quantity of formazan dye

is related directly to the number of metabolically active cells in the medium. Cells (5-7×10^3) were seeded into wells of a 96-well culture plate and incubated with AGE for the times indicated. WST-1 cell proliferation reagent was added directly to the supernatant and incubated at 37℃ for 3hrs. The absorbancy of the solubilized dark red formazan product was then determined at 450nm.

5. Immunoblot analysis

Confluent RAoSMCs were cultured for 48 hrs in serum-free DMEM and were pre-treated with AGE (0-100μg/ml) for 1 day at 37℃. After treatment with different concentration of AGE, cells were rinsed in ice-cold PBS and treated with lysis buffer (1% Triton X-100, 0.1% mercaptoethanol, 1 mM EDTA, 1 mM EGTA, 50 mM Tris-HCl (pH 7.0), 1 mM PMSF) for 20 min on ice. Cell lysates were collected into microcentrifuge tubes, vortexed, and centrifuged at 12,000 rpm for 20 min. Protein concentration was measured in the supernatant using a DC protein assay reagent according to the manufacturer's instructions and equalized for all samples. Reduced samples (30u g) were subjected to SDS–PAGE (NuPAGE™ 4–12% Bis-Tris gel) and then electrotransferred to nitrocellulose membrane. For detection of phosphorylated ERK-1/2, membranes were incubated with antibody directed against a phospho-specific ERK-1/2

followed by incubation with goat anti-rabbit IgG conjugated to horseradish peroxidase. ECL detection method was employed for color development

6. RNA interference

For function-blocking experiments, we used small interfering RNA molecules (siRNA) targeted at RAGE mRNA. A 21-nt sequence for siRNA was derived from the rat RAGE (GenBank accession no. GI: 81722) obtained from Ambion, Inc. (TX, USA): small interfering RNA against RAGE (sense, 5'-GCUAGAAUGGAAACUGAACTT-3'; antisense, 5'-GUUCAGUUUCCA UUCUAGCTT-3'). Smooth muscle cells were transfected with si-RAGE duplexes by using siPORT NeoFX (Ambion Inc. TX. USA). Briefly, RNA duplex (10nmol of final concentration) was incubated in serum-free DMEM containing 15 μl of siPORT NeoFX for 10 min. The complex was added to the empty 60mm culture plate and then overlay smooth muscle cell suspension (1 x 10^5 cells per plate) onto the culture plate wells containing transfection complexes and the transfected cells were incubated in normal cell culture conditions until ready for assay.

7. Immunocytochemistry

Cells were grown on 4-well plastic dishes (SonicSeal Slide, Nalge Nunc, Rochester, NY, USA). Following incubation, The cells were washed twice with PBS and then fixed with 4% paraformaldehyde in 0.5 ml PBS for 30 min at room temperature. The cells were washed again with PBS and then permeabilized for 30 min in PBS containing 0.2% triton. The cells were then blocked in PBS containing 10% goat serum and then incubated for 1 hr with rabbit polyclonal HA antibody. The cells were rewashed three times for 10 min with PBS and incubated with FITC-conjugated goat anti-rabbit antibody as the secondary antibody for 1 hr. Photographs of cells were taken under fluorescence by immunofluorescence microscopy (Olympus, Melville, NY, USA). All images were made by using an excitation filter under reflected light fluorescence microscopy and transferred to a computer equipped with MetaMorph software ver. 4.6 (Universal Imaging Corp. Meraux, LA, USA). All images were made by using an excitation filter under reflected light fluorescence microscopy and transferred to a computer equipped with MetaMorph software ver. 4.6 (Universal Imaging Corp. Meraux, LA, USA).

8. Measurement of intracellular reactive oxygen species generation

RASMCs were labeled with 2′, 7′-dichlorodihydrofluorescein diacetate

(H2DCFDA; Molecular Probe, CA, USA)[14]. The probe H2DCFDA (5M) enters the cell and the acetate group on H2DCFDA is cleaved by cellular esterases, trapping the nonfluorescent 2′, 7′-dichlorofluorescin (DCF) inside. Subsequent oxidation by reactive oxygen species yields the fluorescent product DCF. The dye, when exposed to an excitation wavelength of 480 nm, emits light at 535 nm only when it has been oxidized. Labeled RASMCs were examined using a luminescence spectrophotometer for oxidized dye. The quiescent cells were treated with AGE for 3 h before labeling with H2DCFDA.

9. Statistical analysis

Statistical analyses were performed with SPSS software (SPSS, Chicago, Illinois, USA). To avoid arbitrary cutoff point of the serum AGEs level for prediction of ISR, we performed the receiver operating characteristics (ROC) analysis. Comparison of the continuous variables between the subgroups was performed by the Students t-test. Multivariate analysis (logistic regression) was performed to determine the parameters that predict angiographic ISR. All the tests were two-tailed, and a $p < 0.05$ was considered as significant.

III. **RESULTS**

1. Level of serum AGEs and ISR

The serum levels of AGEs in 203 patients measured in this study ranged from 48.5 U/ml to 283.3 U/ml (151.1±56.5 U/ml, mean±SD). The baseline clinical characteristics of the high and low serum AGE groups were found to be well matched with regard to various parameters other than the duration of diabetes

	Level of AGEs (U/ml)		
	≤ 170 (n=118)	> 170 (n=85)	p
Age (yrs)	60.3 ± 8.6	60.1 ± 8.9	0.870
Male	88 (75)	60 (71)	0.431
Hypertension	41 (35)	32 (38)	0.462
Smoker	47 (40)	33 (39)	0.673
BMI	25.4 ± 2.9	25.9 ± 3.2	0.321
Duration of DM (yrs)	4.7 ± 4.2	9.4 ± 5.8	0.001
HbA1C (%)	7.8 ± 1.5	8.3 ± 1.7	0.044
Hypercholesterolemia	43 (36)	33 (39)	0.310
Low HDL-cholesterol	43 (36)	32 (38)	0.496
Hypertriglyceridemia	32 (27)	25 (29)	0.452
Acute Coronary Syndrome	62 (53)	50 (59)	0.174
Multivessel Disease	98 (66)	82 (71)	0.380
Target Vessel			
LAD	71 (48)	56 (49)	0.713
RCA	43 (29)	27 (23)	0.321
LCX	34 (23)	32 (28)	0.231
B2 or C target lesion	100 (68)	79 (69)	0.846
Stent Length (mm)	19.8 ± 5.8	20.3 ± 5.6	0.561
Pre-PCI MLD (mm)	0.8 ± 0.4	0.7 ± 0.5	0.365

Table 1. Baseline Clinical Characteristics of the Diabetic PatientsValues are n (%) or mean±SD, AGEs=advanced glycation endproducts; BMI=body mass index; DM=diabetes mellitus; $HbA1_C$=hemoglobin $A1_C$; HDL=high density lipoprotein. LAD=left anterior descending coronary artery; LCX=left circumflex artery; MLD=

minimal luminal diameter; PCI=percutaneous coronary intervention; RCA=right coronary artery.

The serum levels of AGEs showed a significant correlation with HbA1C ($r=0.523$, $p=0.001$) and the duration of diabetes ($r=0.415$, $p<0.01$). The QCA data obtained at the baseline and immediately after the procedure were not significantly different between two groups. ROC analysis indicated that the serum level of AGEs had adequate accuracy for the in-stent restenosis. The optimal cutoff point of AGEs was 170.2 U/ml. ROC area was 0.659; 95% CI 0.589 to 0.729; $p < 0.001$ (sensitivity=0.619, specificity=0.659) The six months follow-up angiographic findings showed that the overall ISR rate was 28.9% and the values in the high-AGEs (>170 U/ml) group were 40.1%, whereas the values in the low-AGEs ($\leq$ 170 U/ml) group were 19.6%. The difference in respect of the ISR rate between two groups was statistically significant ($p <0.001$) (Figure 2).

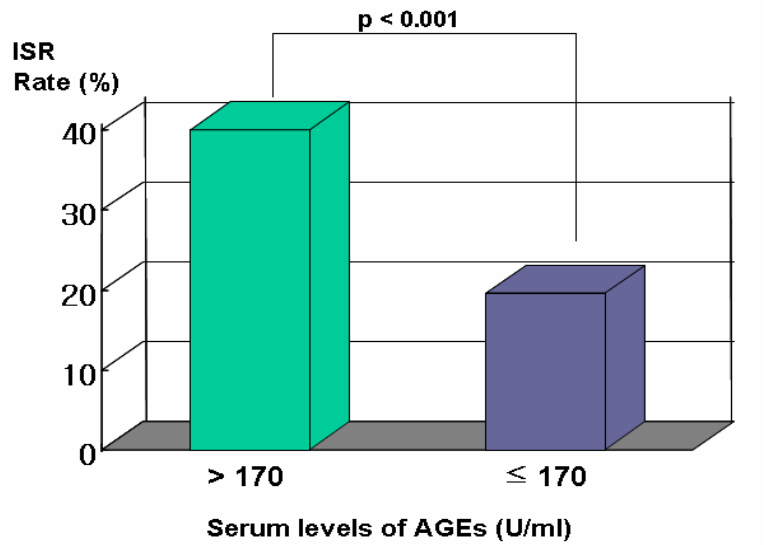


Figure 2. The 6months follow-up angiographic findings showed that the overall ISR rate was 28.9% and the values in the high-AGEs (>170 U/ml) group were 40.1%, whereas the values in the low-AGEs (≤ 170 U/ml) group were 19.6%. The difference in respect of the ISR rate between two groups was statistically significant.

2. Multivariate Analysis

The multivariate analysis of the risk factors for ISR has demonstrated that a high level of serum AGEs (> 170 U/ml) is an independent risk factor for the development of angiographic restenosis. (Odds ratio, 2.659; 95% CI, 1.431-4.940; p=0.002) (Figure 3). Small vessel caliber (post MLD ≤ 3.0 mm) and a long lesion (implanted stent length ≥ 24 mm) were found to be significantly

associated with angiographic ISR.

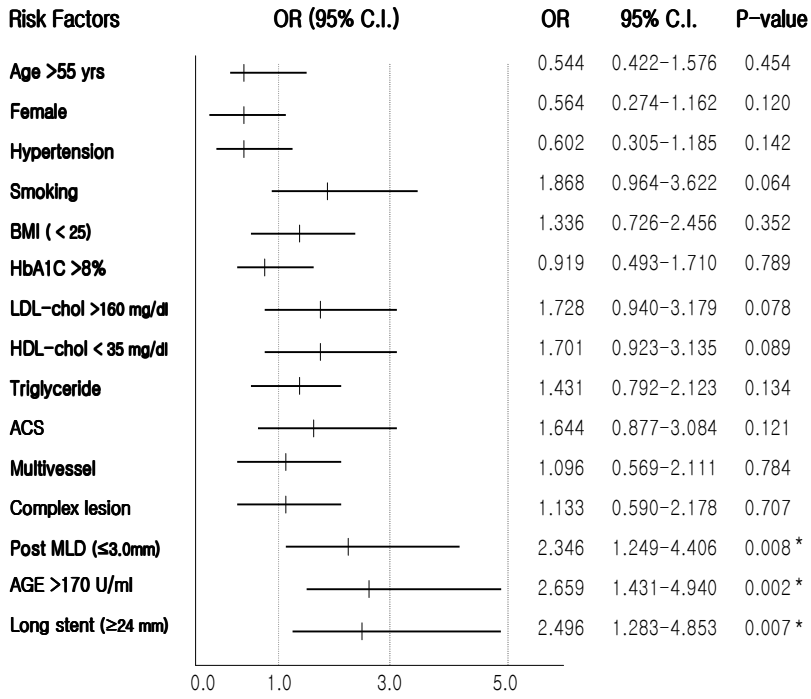
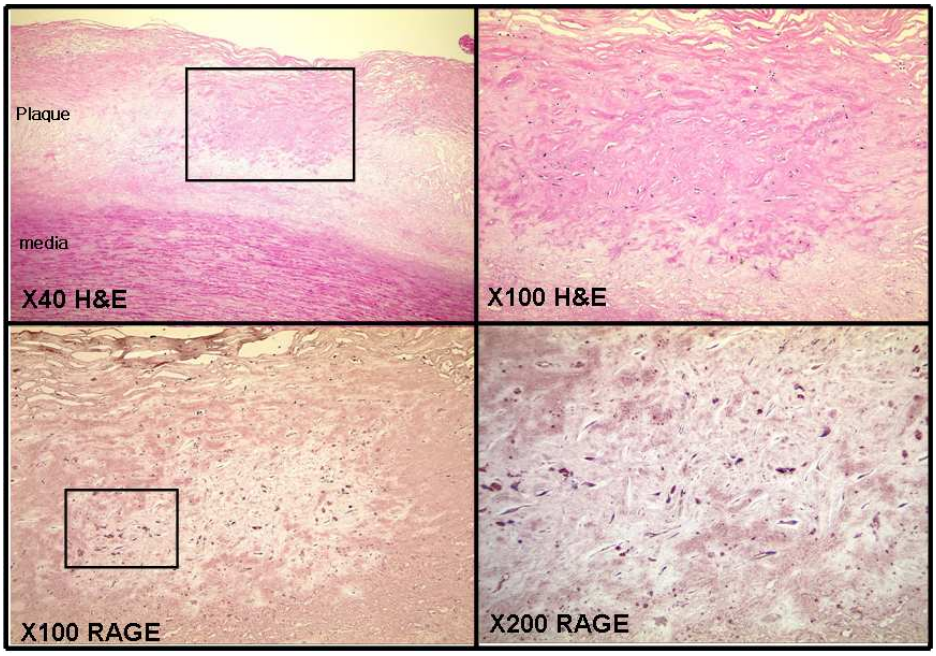


Figure 3. The multivariate analysis of the risk factors for ISR has demonstrated that a high level of serum AGEs is an independent risk factor for the development of angiographic restenosis. (OR, 2.659; 95% CI, 1.431-4.940; p=0.002). Small vessel caliber and a long lesion were found to be significantly associated with angiographic ISR. (Adopted from our previously published data) ACS indicates acute coronary syndrome; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; MLD, minimal luminal diameter.

3. Expression of RAGE in human atheroma

Carotid atheroma specimens showed a thickened intima associated to an area of necrotic core and lipid-laden atheroma in all cases. RAGE was stained as dark brown within atheromatous plaques. RAGE stained cells were distributed mainly in the base of atherosclerotic plaque, in the medio-intimal junction area and its immunoreactivity was colocalized with macrophage, mononuclear cells, and smooth muscle cells. (Figure 4).



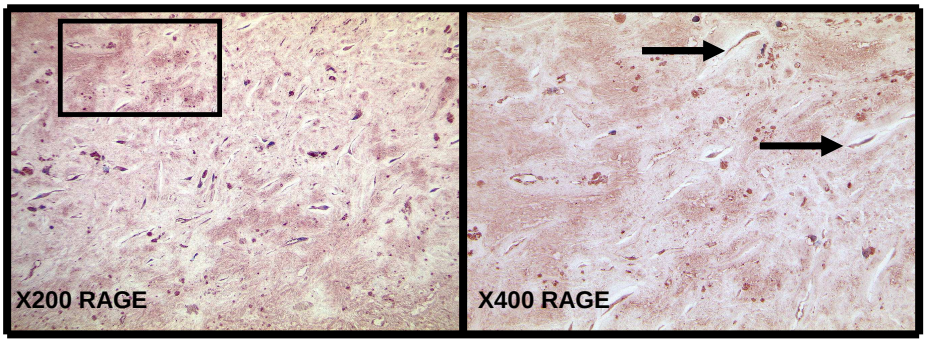


Figure 4. Immunohistochemistry of RAGE in human carotid atheroma. RAGE was stained as dark brown within atheromatous plaques. RAGE stained cells were distributed mainly in the base of atherosclerotic plaque, in the medio-intimal junction area and its immunoreactivity was colocalized with macrophage, mononuclear cells, and smooth muscle cells (arrows).

4. Proliferative effect of AGEs in cultured RAoSMC

Compared with controls AGEs stimulation group showed increased smooth muscle proliferation. RAoSMC proliferation was increased until AGEs concentration 100μg/ml suggest AGEs increase cell proliferation by concentration dependent manner (Figure 5).

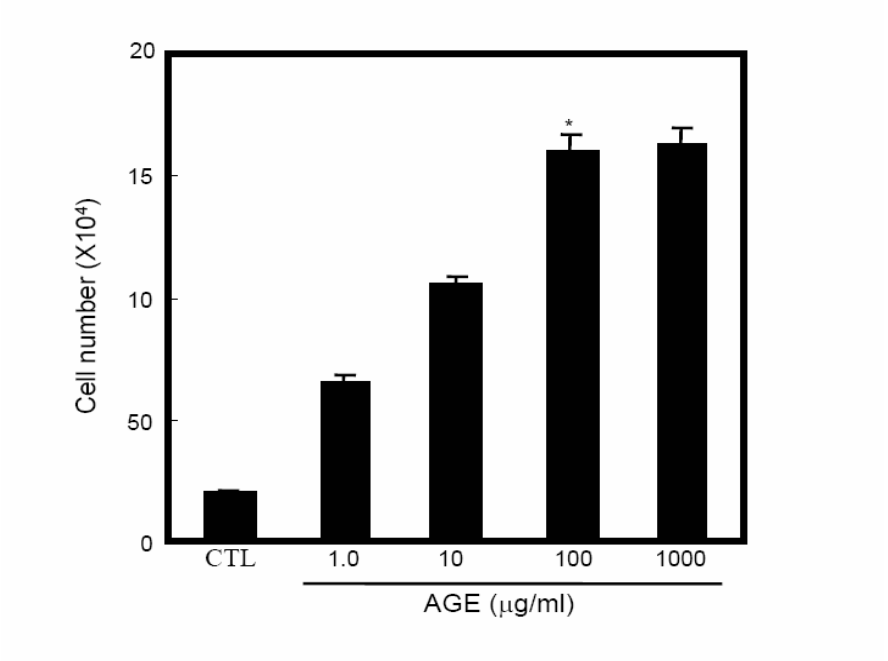


Figure 5. Compared with control AGEs stimulation group showed increased smooth muscle proliferation until AGEs concentration 100 μg/ml by concentration dependent manner.

5. Effects of AGEs on phosphorylation of MAPKs in RAoSMC

Western blot analysis of p-ERK, p-JUN, p-p38 were performed in cultured RAoSMC with or without AGE stimulation. Compared with control group, VSMC cultured with 50mg/mL concentration AGE stimulation revealed increased phosphorylation of MAPK system. Activity of p-ERK and p-p38 was markedly increased in AGE stimulated group comparing with p-JUN (Figure 6).

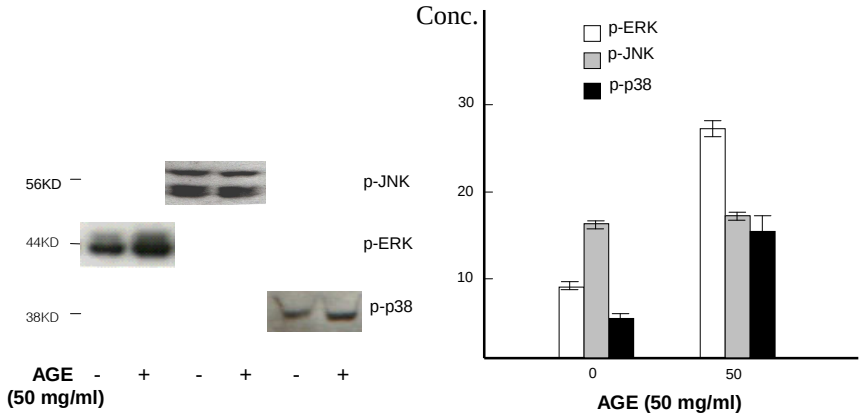


Figure 6. Western blot analysis of p-ERK, p-JUN, p-P38 were performed in cultured RASMC with or without AGE stimulation. Compared with control group, VSMC cultured with 50mg/mL concentration AGE stimulation revealed increased phosphorylation of MAPK system. Activity of p-ERK and p-P38 was markedly increased in AGE stimulated group comparing with p-JUN.

6. Effects of AGEs on phosphorylation of ERK in cultured RAoSMC

Activity of p-ERK was increased in AGEs stimulation group compared with control group. AGEs stimulates phosphorylation of ERK in concentration dependent manner (Figure 7A).

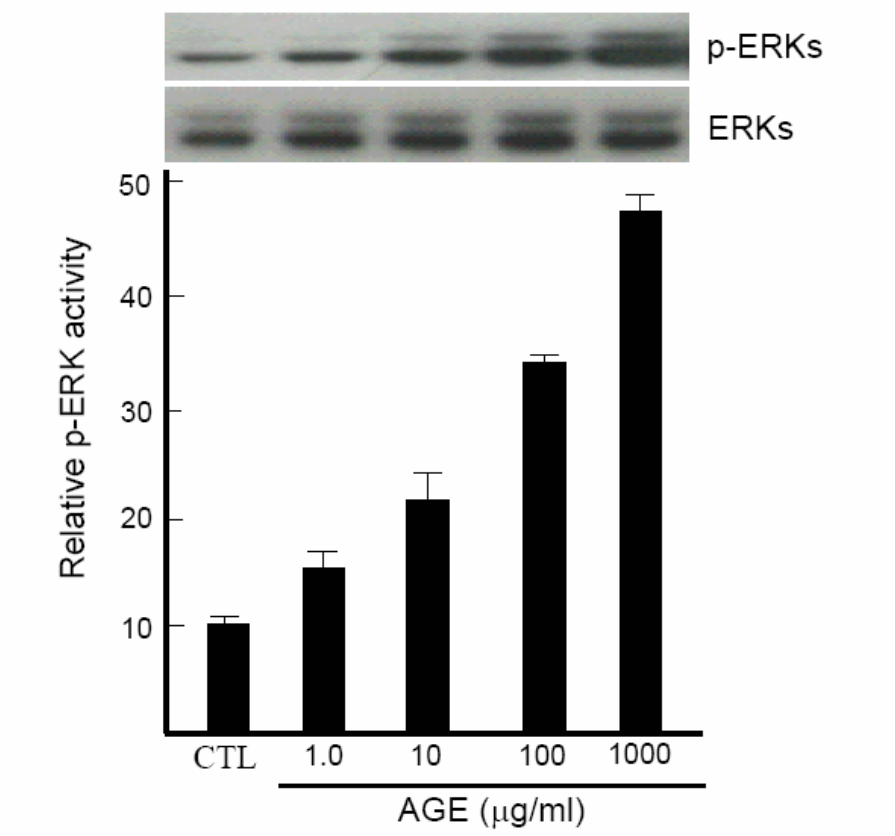


Figure 7A. Activity of p-ERK was increased in AGEs stimulation group compared with control group. AGEs stimulates phosphorylation of ERK in concentration dependent manner.

To determine how AGEs affect ERK activity in various time points up to 12 h after treatment with 100 μg/ml in 0.5% serum-treated RAoSMCs. As shown in Figure 7B, phosphorylation of ERK was increased with the prolonged exposure with the AGEs.

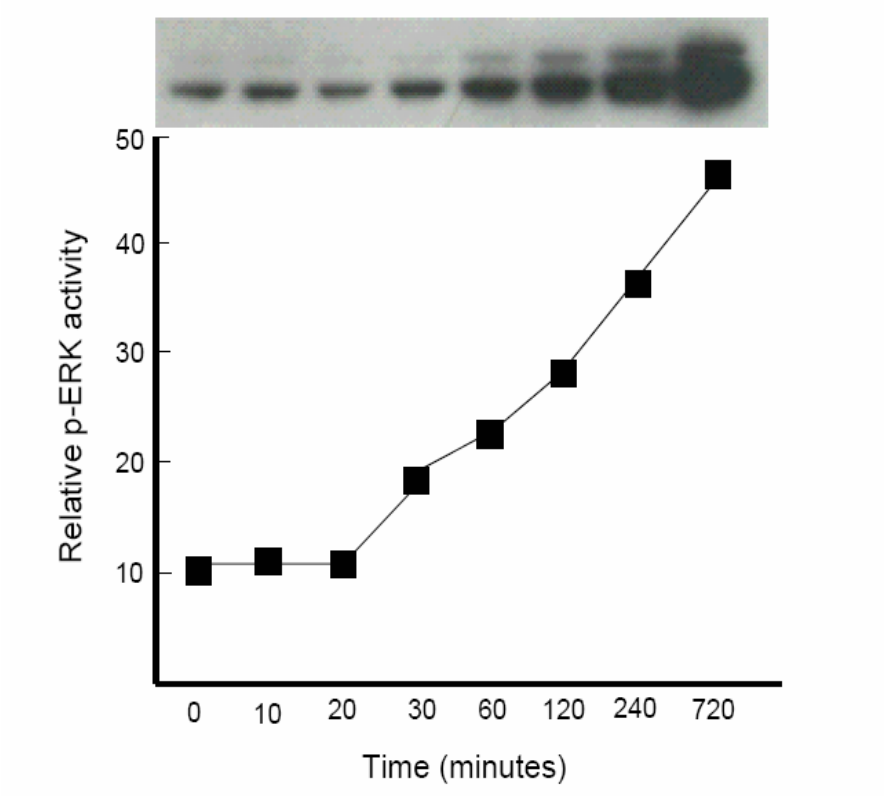


Figure 7B. Phosphorylation of ERK was increased with the prolonged exposure with the AGEs.

To further confirm whether the phosphorylation of ERKs was specific response for AGEs treatment, siRNA for RAGE was treated and activation level of ERKs was estimated. Expression of RAGE protein showed reduction of 90% by RNA interference and was detected by Western blot analysis. The ERKs activation increased by AGEs was decreased by RNA interference for RAGE, indicating that AGEs activated ERKs via RAGE (Figure 7C).

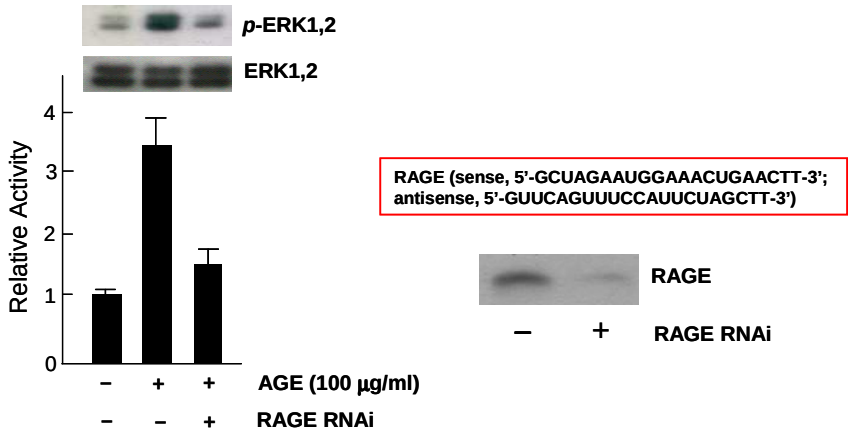


Figure 7C. Inhibition of RAGE expression by siRNA for RAGE attenuated phosphorylation of ERKs by AGE. RASMCs were transfection with siRNA to a final concentration of 10nM or vehicle and stimulated by AGE for 3 h. Each signal was detected by immunoblot analysis using anti-ERK antibody and was quantified by densitometrical analysis.

7. Effects of AGEs on the formation of intracellular ROS

Confocal microscopy of intracellular DCF revealed increased DCF fluorescence with AGE stimulation concentration dependently suggesting AGEs increased intracellular ROS (Figure 8).

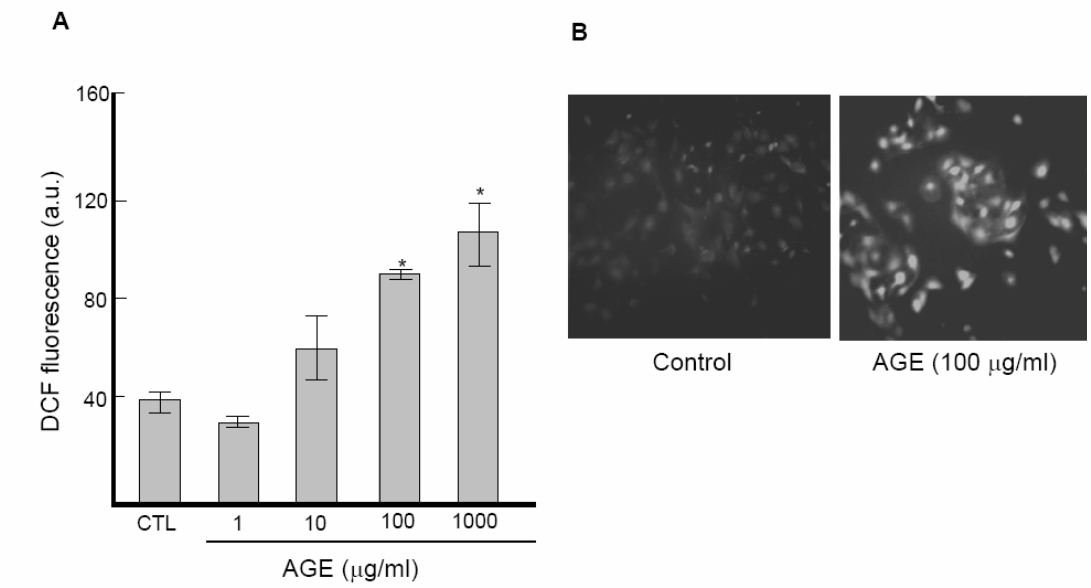


Figure 8. Confocal microscopy of intracellular DCF revealed increased DCF fluorescence with AGE stimulation concentration dependently suggesting AGEs increased intracellular ROS.

IV. DISCUSSION

One of the challenges in cardiovascular medicine is the reducing the complications in patients after PCI, especially for those who with diabetes mellitus, who are particularly prone to ISR. Although ISR have much decreased with the use of in the drug eluting stent (DES) recently, but DES itself has other unexpected complications. It is well known that restenosis and overall consequent adverse cardiac events are more frequent in diabetics compared with

non-diabetics who underwent PCI even with the DES. This finding may reflect differences in the nature of restenosis in this population compared with that in nondiabetic subjects undergoing comparable interventions. Although, a few studies have identified the clinical and angiographic predictors of restenosis in diabetes patients, all the factors relating to the probability of restenosis after stent deployment in this high-risk patients subgroup are not known[15]. Our studies have demonstrated that the ratio of angiographic ISR was significantly elevated in the high serum AGEs group[16]. We also demonstrated basal immunoreactivities of RAGE in human atheroma.

In the setting of hyperglycemia in diabetes, long-term exposure of free amino groups on polypeptides or lipids to higher levels of glucose eventuates in the formation of advanced-glycation end products[17]. AGEs are increased at sites of atherosclerotic lesions, especially in diabetes[18,19]. Increased expression of AGEs has also been found in settings like renal failure and amyloidosis, indicating the biology of AGEs extends beyond diabetes. The cellular effects of AGEs are largely mediated by their specific engagement of cell surface receptor RAGE. Studies have demonstrated RAGE expression at a very low levels in a range of cells, including endothelial and smooth muscle cells and mononuclear cells, RAGE expression increases and receptor upregulation is sustained when

particular pathological processes intervene[20,21].

Several recent experimental studies showed that AGEs can actively participate in neointimal formation after arterial injuries. Zhou et al demonstrated that there was significantly increased accumulation of AGEs and increased immunoreactivities of RAGE and S100/calgranulins in carotid artery of diabetic rats in response to balloon injury compared with that of nondiabetic rats. Blockade of the RAGE/ligand interaction significantly decreased the S100-stimulated vascular smooth muscle cell (VSMC) proliferation in vitro and suppressed neointimal formation, and increased luminal area in both Zucker diabetic and nondiabetic rats[7]. These findings indicate that RAGE/ligand interaction plays a key role in neointimal formation after PCI, especially in diabetics and suggest the plausibility of RAGE blockade as a therapeutic target in vascular injury, both in euglycemia and diabetes.

An increasing body of literature has begun to elucidate the pathobiological mechanisms underlying the RAGE/ligand interaction. AGEs has been shown to induce significant dose-dependent SMC migration[22], and RAGE/ligand interaction upregulates the production of various cytokines and growth factors such as TNF-α[23] and PDGF[24]. SMC migration by AGE was significantly inhibited by an antibody against transforming growth factor-β (TGF-β), and

TGF-β secreted into the culture medium from AGE-stimulated VSMCs was 7-fold higher than that of control, suggesting a potential role for RAGE/ ligand interaction in TGF-β release after vascular injury[22]. In addition, binding of RAGE to its ligand leads to activation of key cell signaling pathways, such as p44/p42 (erk1/erk2), p21ras, MAP kinases, NF-κB, cdc42/rac, and JAK/Stat, thereby reprogramming cellular properties[25,26]. Balloon injury activates the MAPK pathway in diabetics, and hyperinsulinemia activation of the MAPK pathway has been shown to be of importance in the exaggerated neointimal hyperplasia after balloon injury in diabetic animals[27]. Blockade of RAGE/ ligand interaction decreases MAPK activity in cultured SMCs in a concentration-dependent manner[25].

In our study, we demonstrated VSMC proliferation by AGEs stimulation and these increased VSMC proliferation was due to the increased phosphorylation of ERK and p38, which are very important in MAPK signaling pathway. AGEs stimulated VSMC proliferation and MAPKs phosphorylation by time and concentration dependent manner. Serologic study of diabetic PCI patients revealed high serum levels of AGEs which was independent risk factor for ISR were significantly correlated with HbA1C and time duration of diabetes. Considering the in vitro and serologic study results, it can be explained that

restenosis and increased atherosclerosis of the diabetic patients are related the high serum levels of AGEs, which represented time dependent exposure of poor glycemic control.

Recent study revealed that AGEs and RAGE interaction triggers the intracellular reactive oxygen species (ROS). Activated NADPH oxidase is a central target of RAGE and that ROS generated by this mechanism may significantly impact on cellular properties[28]. Our study also revealed concentration dependent increment of intracellular ROS by AGEs stimulation.

V. CONCLUSION

In conclusion, our data suggest a central role for AGEs as a key factor promoting VSMC proliferation and neointimal hyperplasia after stent deployment in diabetic patients. These observations will give a way to the potentially novel target for limiting the development and progression of neointimal hyperplasia by AGEs.

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Abstract (In Korean)

당뇨병성 혈관병증에서 MAPK 의존성 경로를 통한 Advanced glycation endproducts의 병태생물학적 역할

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강태수

Advanced glycation endproducts(AGEs)는 그 수용체를 통하여 당뇨병성 혈관병증에서 면역 반응 및 염증 반응을 유발 시키는 것으로 알려져 있다. 최근 스탠트로 치료한 당뇨병 환자의 관상동맥 질환에서 재발과 관련된 신성내막 증식에 대한 AGEs의 역할에 대한 보고가 되고 있지만 그 기전에 대해서는 아직 명확하게 밝혀지지 않은 상태이다. 이에 우리는 당뇨병성 혈관병증에 대한 AGEs의 병리학적 기전을 알아보고자 하였다. 관상동맥 질환을 가진 당뇨병 환자에서 형광 분석을 사용하여 혈중 AGEs 농도를 확인 하였고 혈중 AGEs 농도가 높은 환자가 스텐트내 재협착의 독립적인 위험인자임을 다중회귀분석을 통하여 확인 하였다. 또한 인체 동맥 경화반을 면역조직 염색 처리하여 AGEs의 수용체 발현을 증명하였다. 그리고

AGEs 자극하에 Rat 대동맥 평활근 세포를 배양하였고 Western blotting으로 mitogen-associated protein kinase(MAPK) system의 활성도를 측정하였다. 또한 RASMC 증식과 JNK, ERK p-38등의 인산화를 통하여 AGEs의 역할을 규명 하였다. AGEs 자극으로 세포내의 활성 산소종의 발생도 확인 하였다. 이에 AGEs는 당뇨병 환자의 관상동맥 질환에서 스텐트내 재협착의 중요한 기전인 혈관 평활근의 증식에 중요한 역할을 확인 하였고, 이 혈관 평활근의 증식은 AGEs에 의한 MAPK system의 활성화를 가능한 기전으로 제시할 수 있다. 이 결과는 AGEs가 스탠트내 재협착의 예방에 새로운 가능성을 보여줄 것으로 생각된다.

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핵심 되는 말: AGEs, MAPK, 스텐트 재협착, 당뇨병성 혈관병증