METABOLISM CLINICAL AND EXPERIMENTAL 60 (2011) 1271–1277

available at www.sciencedirect.com

ScienceDirect

www.metabolismjournal.com

Glucagon-like peptide–1 suppresses advanced glycation end product–induced monocyte chemoattractant protein–1 expression in mesangial cells by reducing advanced glycation end product receptor level

Yuji Ishibashi[a], *Yuri Nishino*[a], *Takanori Matsui*[a], *Masayoshi Takeuchi*[b], *Sho-ichi Yamagishi*[a,*]

[a] *Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume 830-0011, Japan*

[b] *Department of Life Pharmacy, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan*

ARTICLE INFO

Article history:
Received 25 October 2010
Accepted 24 January 2011

ABSTRACT

Advanced glycation end products (AGE) and receptor for AGE (RAGE) interaction elicits reactive oxygen species (ROS) generation and inflammatory reactions, thereby being involved in the development and progression of diabetic nephropathy. Recently, we, along with others, found that glucagon-like peptide–1 (GLP-1), one of the incretins and a gut hormone secreted from L cells in the intestine in response to food intake, could have anti-inflammatory and antithrombogenic properties in cultured endothelial cells. However, the effects of GLP-1 on renal mesangial cells are largely unknown. Therefore, to elucidate the role of GLP-1 in diabetic nephropathy, this study investigated whether and how GLP-1 blocked AGE-induced monocyte chemoattractant protein–1 expression in human cultured mesangial cells. Gene and protein expression was analyzed by quantitative real-time reverse transcription polymerase chain reactions, Western blots, and enzyme-linked immunosorbent assay. The ROS generation was measured with dihydroethidium staining. Glucagon-like peptide–1 receptor (GLP-1R) was expressed in mesangial cells. Glucagon-like peptide–1 inhibited RAGE gene expression in mesangial cells, which was blocked by small interfering RNAs raised against GLP-1R. Furthermore, GLP-1 decreased ROS generation and subsequently reduced monocyte chemoattractant protein–1 gene and protein expression in AGE-exposed mesangial cells. An analogue of cyclic adenosine monophosphate mimicked the effects of GLP-1 on mesangial cells. Our present study suggests that GLP-1 may directly act on mesangial cells via GLP-1R and that it could work as an anti-inflammatory agent against AGE by reducing RAGE expression via activation of cyclic adenosine monophosphate pathway.

Author contributions: Mr Ishibashi, Ms Nishino, Dr Matsui, and Dr Takeuchi conducted the study. Dr Yamagishi designed the study, analyzed the data, and wrote the manuscript.

* *Corresponding author*. Tel.: +81 942 31 7873; fax: +81 942 31 7873.
E-mail address: shoichi@med.kurume-u.ac.jp (S. Yamagishi).

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.01.010

1. Introduction

Reducing sugars can react nonenzymatically with the amino groups of proteins to form Amadori products [1,2]. 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* (AGE) [1,2]. The formation and accumulation of AGE have been known to progress under diabetic conditions [1,2]. There is a growing body of evidence that AGE and receptor for AGE (RAGE) interaction evokes oxidative stress generation and inflammatory reactions, thereby causing progressive alteration in renal architecture and loss of renal function in diabetes [3-8]. Therefore, the AGE-RAGE–induced oxidative stress in the kidney may be a therapeutic target for diabetic nephropathy.

Glucagon-like peptide–1 (GLP-1) is one of the incretins, a gut hormone secreted from L cells in the intestine in response to food intake [9]. Because GLP-1 not only augments glucose-induced insulin release from pancreatic β-cells, but also suppresses glucagon secretion and slows gastric emptying [9], it has been proposed as a potential therapeutic target for the treatment of patients with type 2 diabetes mellitus. The biological actions of GLP-1 on pancreatic cells are mainly mediated by high-affinity receptor for GLP-1 [10]. Because GLP-1 receptor (GLP-1R) is shown to exist in extrapancreatic tissues, including brain, lung, kidney, and heart [11], it is conceivable that GLP-1 could exert diverse biological effects on extrapancreatic tissues. Indeed, we, along with others, have shown that GLP-1 could have anti-inflammatory and antithrombogenic properties in cultured endothelial cells [11-13]. However, the effects of GLP-1 on renal mesangial cells are largely unknown. Therefore, to elucidate the role of GLP-1 in diabetic nephropathy, this study investigated whether and how GLP-1 blocked AGE-induced monocyte chemoattractant protein–1 (MCP-1) expression in human cultured mesangial cells.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) (essentially fatty acid free and essentially globulin free, lyophilized powder), GLP-1 (7-36) amide, and 8-bromo-cyclic adenosine monophosphate (8-Br-cAMP), an analogue of cyclic AMP (cAMP), were purchased from Sigma (St Louis, MO. D-Glyceraldehyde was from Nakalai Tesque (Kyoto, Japan). Antibodies (Abs) directed against human GLP-1R and RAGE were from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Preparation of AGE-BSA

The AGE-BSA was prepared as described previously [14]. In brief, BSA (25 mg/mL) was incubated under sterile conditions with 0.1 mol/L glyceraldehyde in 0.2 mol/L $NaPO_4$ buffer (pH 7.4) for 7 days. Unincorporated sugars were then removed by PD-10 column chromatography and dialysis against phosphate-buffered saline. Control nonglycated BSA was incubated in the same conditions except for the absence of reducing sugars. Preparations were tested for endotoxin using Endospecy ES-20S system (Seikagaku, Tokyo, Japan); no endotoxin was detectable. The extent of chemical modification was determined as described with 2,4,6-trinitrobenzenesulfonic acid as a difference in lysine residues of modified and unmodified protein preparations. The extent of lysine modification (percentage) of modified BSA preparations was 65% for AGE-BSA.

2.3. Cells

Mesangial cells from human kidney (supplied by Clonetics, San Diego, CA) were maintained in mesangial basal medium supplemented with 5% fetal bovine serum according to the supplier's instructions. The AGE and/or GLP-1 treatments were carried out in a medium containing 0.5% fetal bovine serum.

2.4. Construction and transfection of small interfering RNAs

The sense and antisense human GLP-1R small interfering RNAa (siRNAs) used in this experiment (5′-UCAUCAAGCUGUUUACAGAtt-3′ and 5′-UCUGUAAACAGCUUGAUGAag, respectively) were synthesized by Applied Biosystems (Foster City, CA). Control nonsilencing siRNAs were also obtained from Applied Biosystems (Silencer Negative Control #1 siRNA). Afterward, the siRNA duplexes were transfected to mesangial cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. After 2 days of transfection, GLP-1R protein level was analyzed.

2.5. Immunostaining

Mesangial cells were fixed with 4% paraformaldehyde and washed with phosphate-buffered saline. The cells were stained with mouse Ab raised against α-smooth muscle actin (PROGEN, Heidelberg, Germany). α-Smooth muscle actin was visualized with Alexa Fluor 488 donkey anti–mouse immunoglobulin G (Invitrogen). The cells were imaged under a laser-scanning confocal microscope.

2.6. Western blotting analysis

After transfection, proteins were extracted from mesangial cells with lysis buffer, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes as described previously [14]. Membranes were probed with Abs against GLP-1R or RAGE, and then immune complexes were visualized with an enhanced chemiluminescence detection system (Amersham Bioscience, Buckinghamshire, United Kingdom).

2.7. Real-time reverse transcription polymerase chain reactions

Mesangial cells were treated with 100 μg/mL AGE-BSA or nonglycated BSA in the presence or absence of the indicated concentrations of GLP-1 or 5 μmol/L 8-Br-cAMP for 4 hours.

Total RNA was then extracted with RNAqueous-4PCR kit (Ambion, Austin, TX) according to the manufacturer's instructions. Quantitative real-time reverse transcription polymerase chain reaction was performed using Assay-on-Demand and TaqMan 5 fluorogenic nuclease chemistry (Applied Biosystems) according to the supplier's recommendation. IDs of primers for human RAGE, MCP-1, and β-actin gene were Hs00153957_m1, Hs00234140_m1, and Hs99999903_m1, respectively.

2.8. Dihydroethidium staining

Mesangial cells were treated with 100 μg/mL AGE-BSA or nonglycated BSA in the presence or absence of the indicated concentrations of GLP-1 or 5 μmol/L 8-Br-cAMP for 4 hours, and then the cells were incubated with phenol red free Dulbecco modified Eagle medium containing 3 μmol/L dihydroethidium (Molecular Probes, Eugene, OR). After 15 minutes, the cells were imaged under a laser-scanning confocal microscope. Intensity of dihydroethidium staining in 5 different fields of each sample was analyzed by microcomputer-assisted ImageJ.

2.9. Measurement of MCP-1

The MCP-1 proteins released into media were measured with an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

2.10. Statistical analysis

All values were presented as mean ± SEM. One-way analysis of variance followed by the Scheffe *F* test was performed for statistical comparisons; $P < .05$ was considered significant.

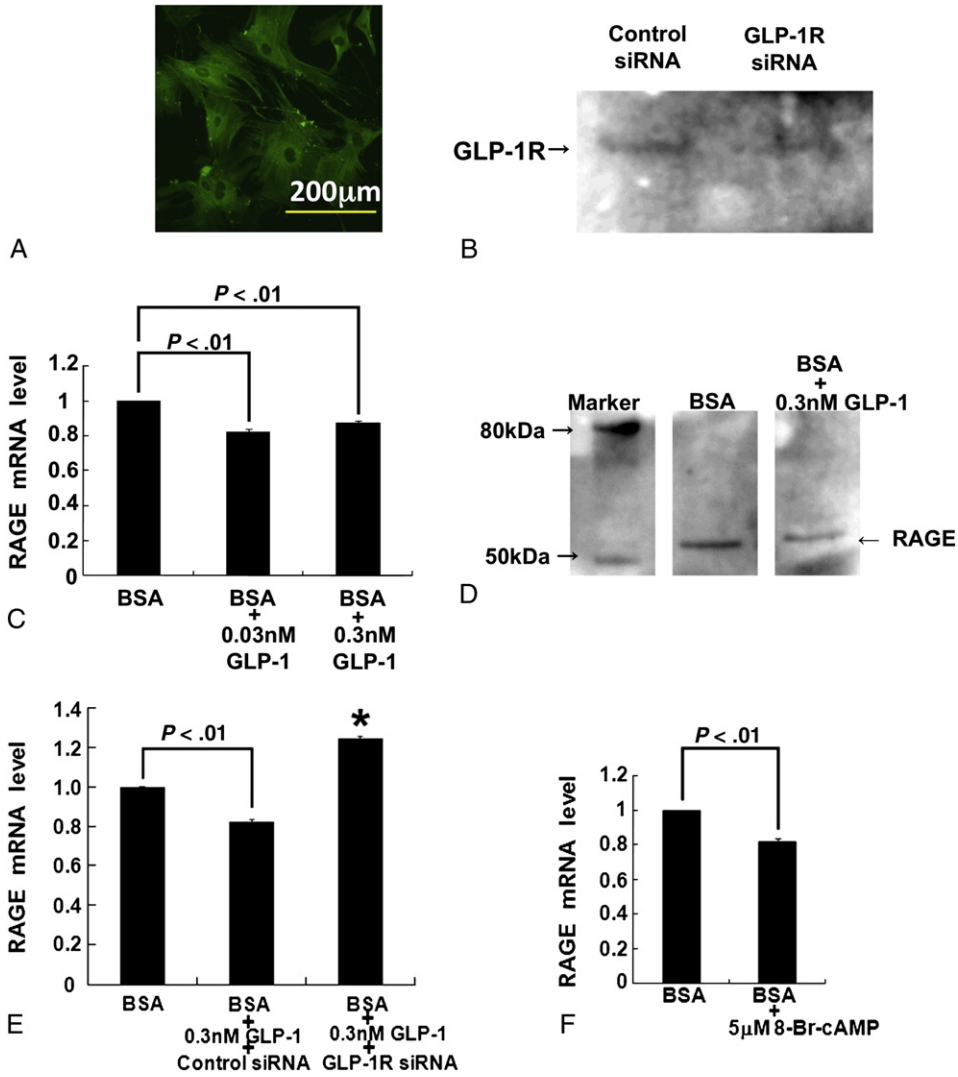

Fig. 1 – The GLP-1R and RAGE expression levels in mesangial cells. A, Immunostaining for α-smooth muscle actin. Transfected or nontransfected mesangial cells were treated with or without 100 μg/mL nonglycated BSA in the presence or absence of the indicated concentrations of GLP-1 or 5 μmol/L 8-Br-cAMP for 4 hours. Afterward, GLP-1R protein (B) and RAGE gene (C, E, and F) and protein levels (D) were measured. B and D, Representative bands of Western blot. *P < .01 compared with the value with BSA + GLP-1 + control siRNA. n = 3 to 6.

3. Results

As shown in Fig. 1A, cells used in the present experiments were positive for α-smooth muscle actin staining. Therefore, we confirmed that they had myofibroblast phenotype.

Because of the ambiguity as to the location of GLP-1R in human kidney, we examined whether mesangial cells expressed GLP-1R. As shown in Fig. 1B, Western blotting analysis revealed a single band with a molecular mass of ca 56 kd, corresponding to the GLP-1R seen in mesangial cells. We also confirmed that siRNA molecules specific for human GLP-1R actually reduced GLP-1R level in mesangial cells to about one third of those of control siRNA-treated cells (Fig. 1B).

Engagement of RAGE with AGE activates its downstream signaling via reactive oxygen species (ROS) generation in various types of cells [3-8]. Therefore, we examined the effect of GLP-1 on RAGE gene expression in mesangial cells. As shown in Fig. 1C, 0.03 and 0.3 nmol/L GLP-1 decreased RAGE mRNA levels to about 80% of control cells. Glucagon-like peptide–1 at 0.3 nmol/L was also found to decrease RAGE protein level to about 80% of control cells (Fig. 1D).

We next examined the involvement of GLP-1R in GLP-1 actions on mesangial cells. For this, we investigated the effects of siRNAs raised against GLP-1R on RAGE gene expression in mesangial cells. Glucagon-like peptide–1 also significantly decreased RAGE gene expression in BSA plus control siRNA-transfected mesangial cells, which was completely prevented by the treatment with GLP-1R siRNA transfection (Fig. 1E). Because the actions of the GLP-1R are mediated by cAMP production and subsequent protein kinase A activation [15], we further studied the effects of an analogue of cAMP, 8-Br-cAMP, on RAGE gene expression in mesangial cells. As shown in Fig. 1F, 8-Br-cAMP significantly reduced RAGE mRNA levels in mesangial cells.

We next investigated whether GLP-1 could actually inhibit RAGE downstream signaling pathway in mesangial cells. For this, we studied the effect of GLP-1 on ROS generation and MCP-1 gene expression in AGE-exposed mesangial cells. As shown in Figs. 2 and 3A, B, GLP-1 or 8-Br-cAMP inhibited the AGE-induced ROS generation and MCP-1 gene expression in mesangial cells. Glucagon-like peptide–1 receptor siRNA, but not control siRNA, significantly inhibited the GLP-1–induced down-regulation of MCP-1 mRNA levels in AGE-exposed mesangial cells (Fig. 3A). Furthermore, AGE significantly increased MCP-1 production by mesangial cells, which was blocked by the treatment with GLP-1 (Fig. 3C).

4. Discussion

In the present study, we demonstrated for the first time that GLP-1 reduced RAGE mRNA and protein levels and inhibited the AGE-induced oxidative stress generation and MCP-1 expression in human cultured mesangial cells. We have previously shown that MCP-1 gene induction in AGE-exposed mesangial cells is suppressed by diphenylene iodonium or pyrrolidine dithiocarbamate, an inhibitor of NADPH oxidase or nuclear factor–κB, respectively [8,16]. Furthermore, in this study, the magnitude of increase in MCP-1 mRNA level induced by AGE was similar to that in ROS generation (Figs. 2A and 3A). Given that RAGE is a major receptor that mediates various biological actions of AGE [3-8], our present study suggests that GLP-1 inhibits the AGE-elicited MCP-1 gene and protein expression in mesangial cells by reducing RAGE level and subsequently suppressing NADPH oxidase-mediated ROS generation and nuclear factor–κB activation.

In this study, we found that GLP-1R was expressed in mesangial cells and that GLP-1R siRNAs, but not control siRNAs, inhibited the GLP-1–induced down-regulation of RAGE mRNA levels. These observations suggest that control siRNAs themselves could not have specific effects on RAGE gene expression in mesangial cells. Moreover, an analogue of cAMP, 8-Br-cAMP, mimicked the effects of GLP-1 on RAGE gene expression, ROS generation, and MCP-1 mRNA levels in mesangial cells. Because GLP-1R mainly mediates the biological actions of GLP-1 on pancreatic cells via cAMP pathway [10,15], our present results suggest that antioxidative and anti-inflammatory actions of GLP-1 on mesangial cells are partly mediated by cAMP pathway via the interaction with GLP-1R. We have previously shown that AGE decrease intracellular cAMP levels in human cultured endothelial cells and that cAMP agonists such as dibutyryl cAMP reduce the AGE-RAGE–stimulated endothelial plasminogen activator inhibitor–1 production [17]. Furthermore, beraprost sodium, an active prostacyclin analogue with cAMP-elevating activity, was reported to down-regulate RAGE mRNA levels in AGE-exposed pericytes, a counterpart of mesangial cells in microvessels [18,19]. Taken together, these findings suggest the active involvement of cAMP in the AGE-signaling pathways and that RAGE is a molecular target for anti-inflammatory effects of GLP-1–GLP-1R–cyclic AMP axis in AGE-exposed mesangial cells.

Monocyte chemoattractant protein–1 plays an important role in the early phase of diabetic nephropathy by initiating monocyte recruitment to the mesangial areas [20]. Indeed, plasma MCP-1 levels are associated with albumin excretion rate in patients with type 1 diabetes mellitus, a marker of early diabetic nephropathy [21]. Furthermore, selective targeting of MCP-1 was shown to markedly decrease albuminuria, renal injury, and fibrosis in streptozotocin-induced diabetic nephropathy [22]. Therefore, pharmacological up-regulation or substitution of GLP-1 may play a protective role against diabetic nephropathy by suppressing MCP-1 expression via blockade of the deleterious effects of AGE. In this regard, strategies to enhance the biological actions of GLP-1, including GLP-1 derivatives or dipeptidyl peptidase–4 inhibitors, may be promising for not only ameliorating hyperglycemia, but also protecting against renal injury in type 2 diabetes mellitus patients because the effects of GLP-1 are attenuated in these subjects [23].

The plasma concentration of GLP-1 in type 2 diabetes mellitus patients is reported to be about 0.01 to 0.025 nmol/L [24]. However, treatment with dipeptidyl peptidase–4 inhibitors has been shown to increase its plasma level by approximately 2- to 4-fold [24,25]. In addition, administration of GLP-1 derivatives could dramatically increase its biological activity in humans [26]. Therefore, the concentration of GLP-1 having beneficial effects on mesangial cells (0.03-0.3 nmol/L) may be comparable to the therapeutic levels, which are

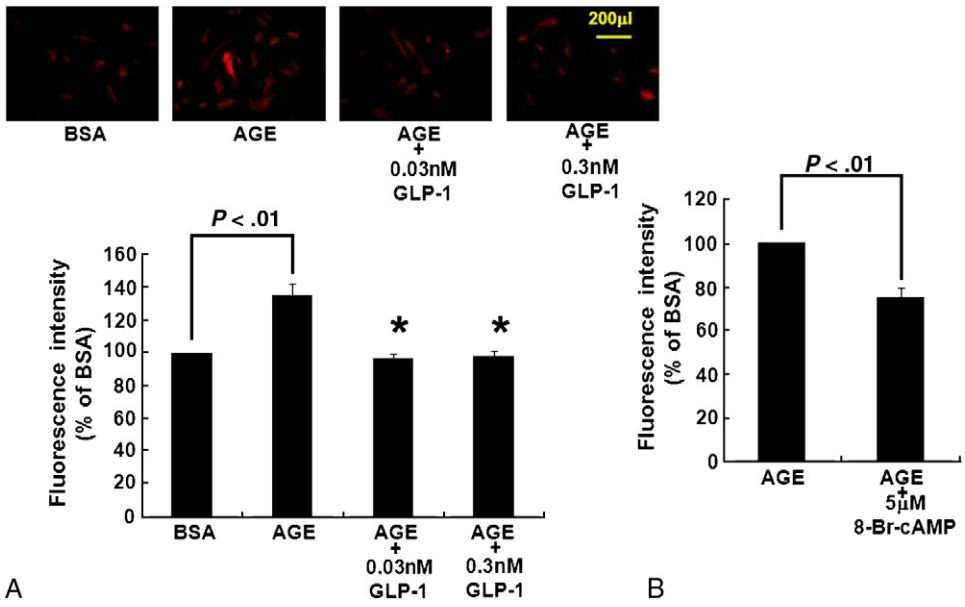

Fig. 2 – Effects of GLP-1 on ROS generation in mesangial cells. Mesangial cells were treated with 100 μg/mL AGE-BSA or nonglycated BSA in the presence or absence of the indicated concentrations of GLP-1 (A) or 5 μmol/L 8-Br-cAMP (B) for 4 hours. Afterward, ROS generation was measured. A, Upper panel shows the representative microphotographs. Lower panel shows the quantitative data. *P < .01 compared with the value with AGE alone. n = 3.

achieved in the treatments of patients with type 2 diabetes mellitus. Furthermore, in this study, in vitro–modified AGE were prepared by incubating BSA with glyceraldehyde for 1 week; this process produced relatively highly modified proteins in comparison to those in vivo. However, it is unlikely that extensively modified, unphysiologic AGE that were

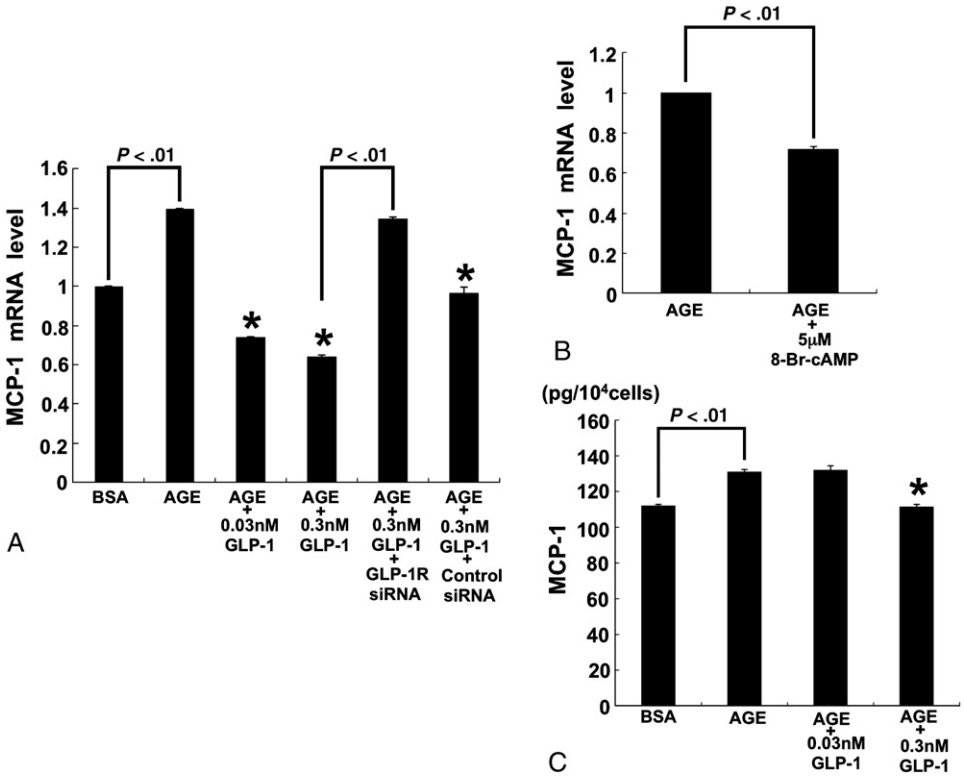

Fig. 3 – Effects of GLP-1 on MCP-1 gene (A and B) and protein (C) expression in mesangial cells. Transfected or nontransfected mesangial cells were treated with 100 μg/mL AGE-BSA or nonglycated BSA in the presence or absence of the indicated concentrations of GLP-1 (A and C) or 5 μmol/L 8-Br-cAMP (B) for 4 hours. Afterward, MCP-1 gene (A and B) and protein (C) expression levels were measured. *P < .01 compared with the value with AGE alone. n = 3.

formed under the in vitro conditions may exert nonspecific and toxic effects on mesangial cells for the following reasons: (1) we have previously found that immunological epitope of glyceraldehyde-modified AGE was actually present in serum of diabetic patients and that the concentration (100 μg/mL) of in vitro–prepared AGE used here were comparable with that of the in vivo diabetic situation; (2) we have also shown previously that the AGE-rich serum fractions obtained from diabetic patients on hemodialysis have the same biological effects as did the in vitro–prepared AGE; and (3) preincubation of AGE-containing media with 1 μg/mL polymyxin B, an inhibitor of endotoxin, for 30 minutes did not affect the AGE-induced ROS generation [14,27-29].

5. Limitations

In this study, we found that siRNAs raised against GLP-1R, but not control siRNAs, inhibited the GLP-1–induced down-regulation of RAGE and MCP-1 mRNA levels in AGE-exposed mesangial cells, thus suggesting that the biological actions of GLP-1 could be mediated through the interaction with GLP-1R. However, we cannot totally exclude the possibility that GLP-1 and GLP-1 degradation products may also exert protective actions in mesangial cells through GLP-1R independent pathways.

Furthermore, it would be interesting to examine whether long-term treatment with GLP-1 could ultimately down-regulate GLP-1R expression in mesangial cells and subsequently attenuate its beneficial action on diabetic nephropathy.

Acknowledgment

This work was supported in part by Grants of Collaboration with Venture Companies Project from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (SY).

REFERENCES

[1] Vlassara H, Bucala R. Recent progress in advanced glycation and diabetic vascular disease: role of advanced glycation end product receptors. Diabetes Suppl 1996;3:S65-6.
[2] Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1998;318:1315-21.
[3] Yamagishi S, Imaizumi T. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. Curr Pharm Des 2005;11:2279-99.
[4] Hudson BI, Bucciarelli LG, Wendt T, et al. Blockade of receptor for advanced glycation end products: a new target for therapeutic intervention in diabetic complications and inflammatory disorders. Arch Biochem Biophys 2003;419:80-8.
[5] Yamagishi S, Matsui T, Nakamura K. Kinetics, role and therapeutic implications of endogenous soluble form of receptor for advanced glycation end products (sRAGE) in diabetes. Curr Drug Targets 2007;8:1138-43.
[6] Wendt TM, Tanji N, Guo J, et al. RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. Am J Pathol 2003; 162:1123-37.
[7] Yamamoto Y, Kato I, Doi T, et al. Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. J Clin Invest 2001;108:261-8.
[8] Ide Y, Matsui T, Ishibashi Y, Takeuchi M, Yamagishi S. Pigment epithelium-derived factor inhibits advanced glycation end product–elicited mesangial cell damage by blocking NF-kappaB activation. Microvasc Res 2010;80: 227-32.
[9] Kim W, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. Pharmacol Rev 2008;60:470-512.
[10] Winzell MS, Ahrén B. G-protein–coupled receptors and islet function—implications for treatment of type 2 diabetes. Pharmacol Rev 2007;116:437-48.
[11] Liu H, Hu Y, Simpson RW, Dear AE. Glucagon-like peptide–1 attenuates tumour necrosis factor-alpha–mediated induction of plasmogen activator inhibitor–1 expression. J Endocrinol 2008;196:57-65.
[12] Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S. Glucagon-like peptide–1 (GLP-1) inhibits advanced glycation end product (AGE)–induced up-regulation of VCAM-1 mRNA levels in endothelial cells by suppressing AGE receptor (RAGE) expression. Biochem Biophys Res Commun 2010;391: 1405-8.
[13] Liu H, Dear AE, Knudsen LB, Simpson RW. A long-acting glucagon-like peptide–1 analogue attenuates induction of plasminogen activator inhibitor type–1 and vascular adhesion molecules. J Endocrinol 2009;201:59-66.
[14] Yoshida T, Yamagishi S, Nakamura K, et al. Telmisartan inhibits AGE-induced C-reactive protein production through downregulation of the receptor for AGE via peroxisome proliferator-activated receptor–gamma activation. Diabetologia 2006;49:3094-9.
[15] Kieffer TJ, Habener JF. The glucagon-like peptides. Endocr Rev 1999;20:876-913.
[16] Matsui T, Yamagishi S, Nakamura K, Takeuchi M, Inoue H. Nifedipine, a calcium-channel blocker, inhibits advanced glycation end-product–induced expression of monocyte chemoattractant protein–1 in human cultured mesangial cells. J Int Med Res 2007;35:107-12.
[17] Yamagishi S, Fujimori H, Yonekura H, Yamamoto Y, Yamamoto H. Advanced glycation end products inhibit prostacyclin production and induce plasminogen activator inhibitor–1 in human microvascular endothelial cells. Diabetologia 1998;41:1435-41.
[18] Yamagishi S, Amano S, Inagaki Y, Okamoto T, Takeuchi M, Makita Z. Beraprost sodium, a prostaglandin I2 analogue, protects against advanced glycation end products–induced injury in cultured retinal pericytes. Mol Med 2002;8:546-50.
[19] Yamagishi S, Inagaki Y, Okamoto T, et al. Advanced glycation end product–induced apoptosis and overexpression of vascular endothelial growth factor and monocyte chemoattractant protein–1 in human-cultured mesangial cells. J Biol Chem 2002;277:20309-15.
[20] Banba N, Nakamura T, Matsumura M, et al. Possible relationship of monocyte chemoattractant protein–1 with diabetic nephropathy. Kidney Int 2000;58:684-90.
[21] Chiarelli F, Cipollone F, Mohn A, et al. Circulating monocyte chemoattractant protein–1 and early development of nephropathy in type 1 diabetes. Diabetes Care 2002;25: 1829-34.
[22] Chow FY, Nikolic-Paterson DJ, Ozols E, et al. Monocyte chemoattractant protein–1 promotes the development of diabetic renal injury in streptozotocin-treated mice. Kidney Int 2006;69:73-80.
[23] Pratley RE, Gilbert M. Targeting incretins in type 2 diabetes: role of GLP-1 receptor agonists and DPP-4 inhibitors. Rev Diabet Stud 2008;5:73-94.
[24] Herman GA, Bergman A, Stevens C, et al. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase–4 inhibitor, on

incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. J Clin Endocrinol Metab 2006;91:4612-9.

[25] Sarashina A, Sesoko S, Nakashima M, et al. Linagliptin, a dipeptidyl peptidase–4 inhibitor in development for the treatment of type 2 diabetes mellitus: a phase I, randomized, double-blind, placebo-controlled trial of single and multiple escalating doses in healthy adult male Japanese subjects. Clin Ther 2010;32:1188-204.

[26] Elbrønd B, Jakobsen G, Larsen S, et al. Pharmacokinetics, pharmacodynamics, safety, and tolerability of a single-dose of NN2211, a long-acting glucagon-like peptide 1 derivative, in healthy male subjects. Diabetes Care 2002;25:1398-404.

[27] Yamagishi S, Nakamura K, Matsui T, et al. Pigment epithelium-derived factor inhibits advanced glycation end product–induced retinal vascular hyperpermeability by blocking reactive oxygen species–mediated vascular endothelial growth factor expression. J Biol Chem 2006;281: 20213-20.

[28] Takeuchi M, Makita Z, Yanagisawa K, Kameda K, Koike T. Detection of noncarboxymethyllysine and carboxymethyllysine advanced glycation end products (AGE) in serum of diabetic patients. Mol Med 1999;5:393-405.

[29] Takeuchi M, Yanase Y, Matsuura N, et al. Immunological detection of a novel advanced glycation end-product. Mol Med 2001;7:783-91.