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Advanced Glycation End Products Inhibit Production and Activity of Matrix Metalloproteinase-2 in Human Umbilical Vein Endothelial Cells

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The aim of this study was to investigate the effects of advanced glycation end products (AGEs) on the expression and activity of matrix metalloproteinases-2 (MMP-2) in human umbilical vein endothelial cells (HUVECs). Cultured HUVECs were incubated with various concentrations of AGEs-modified albumin or unmodified albumin for different time periods. Protein and gene expression of MMP-2 and the receptor for AGEs (RAGE) were measured by Western blot and reverse transcription-polymerase chain reaction, respectively. The activity of MMP-2 in the conditioned medium was measured by gelatin zymography. The AGE-modified albumin inhibited MMP-2 but increased RAGE protein and gene expression in HUVECs in a concentration- and time-dependent manner. An inhibition of MMP-2 activity was also detected in the conditioned medium of HUVECs incubated with AGEs-modified albumin. In conclusion, AGEs inhibited the expression and activity of MMP-2 in HUVECs; this may be mediated through upregulation of RAGE.

KEY WORDS: Advanced glycation end products (AGEs); Receptor for advanced glycation end products (RAGE); Matrix metalloproteinase-2 (MMP-2); Human umbilical vein endothelial cells (HUVECs)

Introduction

Advanced glycation end products (AGEs) are non-enzymatic, glycated products of proteins, lipids and DNA. Production of AGEs is greatly accelerated in diabetes and this has been recognized as one of the main factors associated with endothelial dysfunction and diabetes complications.[1 – 4] Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases able to degrade extracellular matrix (ECM), the levels of which are closely related to ECM turnover. MMP-2, a gelatinase predominantly secreted by endothelial cells, is involved in the degradation of type IV collagen, the main component of subendothelial basement membrane.[5] The integrity of the basement membrane is very important in maintaining endothelial function. It has been reported that AGEs

influence the production and activity of MMP-2 in various cells,[3,6] but their effects on MMP-2 in vascular endothelial cells have not been examined. This study investigated the effects of AGEs on the production and activity of MMP-2, and on the receptor for AGEs (RAGE), in human umbilical vein endothelial cells in order to explore a mechanism through which AGEs may promote endothelial dysfunction.

Materials and methods

SYNTHESIS OF AGES

The AGEs were synthesized under sterile conditions by incubating 20 mg/ml low endotoxin bovine serum albumin (BSA) (Merck, San Diego, CA, USA) with 50 mmol/l glucose for 90 days as previously reported,[1] and then fully dialysing against phosphate-buffered saline to remove unbound glucose. As a negative control of AGEs, BSA was incubated without glucose under the same conditions.

CELL CULTURE

Primary human umbilical vein endothelial cells (HUVECs) were cultured to 80% confluence (three to four passages) as described previously.[1] They were exposed to: various concentrations of AGEs (0, 0.05, 0.1, 0.2 or 0.4 mg/ml) or to the negative control at a concentration of 0.4 mg/ml for 24 h (control); and to 0.2 mg/ml AGEs for 0, 6, 12, 18 or 24 h or to 0 mg/ml AGEs for 24 h (24 h control). Conditioned serum-free medium from each of the incubated samples was collected for gelatin zymography.

WESTERN BLOT

The levels of MMP-2 and RAGE protein in the HUVECs were analysed by Western blot using rabbit anti-human MMP-2 (1:400, Chemicon, Billerica, MA, USA) and mouse anti-human RAGE antibody (1:1500, Chemicon) as previously described.[7]

REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION

Total RNA extraction (Trizol® Reagent, Invitrogen, Carlsbad, CA, USA) and reverse transcription (Superscript™ III Reverse Transcriptase, including Superscript™ III RT (200 U/µl), 5× first-strand buffer, 0.1 mol/l dithiothreitol, Invitrogen) were performed according to the manufacturer's instructions. The following primers and polymerase chain reaction conditions were used: MMP-2, 563 bp, *5′*-GGATGATGCCTTTGCTCG-3′, 3′-CTGA TGCTGGCGCTGTTCT-*5′*, 30 cycles at 60 °C; RAGE, 372 bp, *5′*-CTGGTGTTCCCAATAAGG-3′, 3′-TTGGCATTGGGACTGGA-*5′*, 38 cycles at 58.5 °C; glyceraldehyde-3-phosphate dehydrogenase (GAPDH; reference gene), 452 bp, *5′*-ACCACAGTCCATGCCATCAC-3′, 3′-ATGTCGTTGTCCCACCACCT-*5′*, 26 cycles at 56 °C.

ZYMOGRAPHY

Gelatin zymography was used to measure the activity of MMP-2 in the conditioned medium, as previously reported.[8] Medium of SMMC-7721, a hepatoma carcinoma cell line, was used as the positive control. The area digested by MMP-2 was quantified with Image J version 1.34 (National Institutes of Health, Bethesda, MD, USA).

STATISTICAL ANALYSIS

All results were expressed as the mean ± SD. Data were analysed using analysis of variance and subjected to *post hoc* comparisons using Dunnett's test (SPSS® statistical software, SPSS Inc., Chicago, IL, USA). A P-value < 0.05 (two-tailed) was considered to be statistically significant.

Results

EFFECTS ON MMP-2 AND RAGE PROTEIN AND GENE EXPRESSION

The effects of AGEs on MMP-2 and RAGE

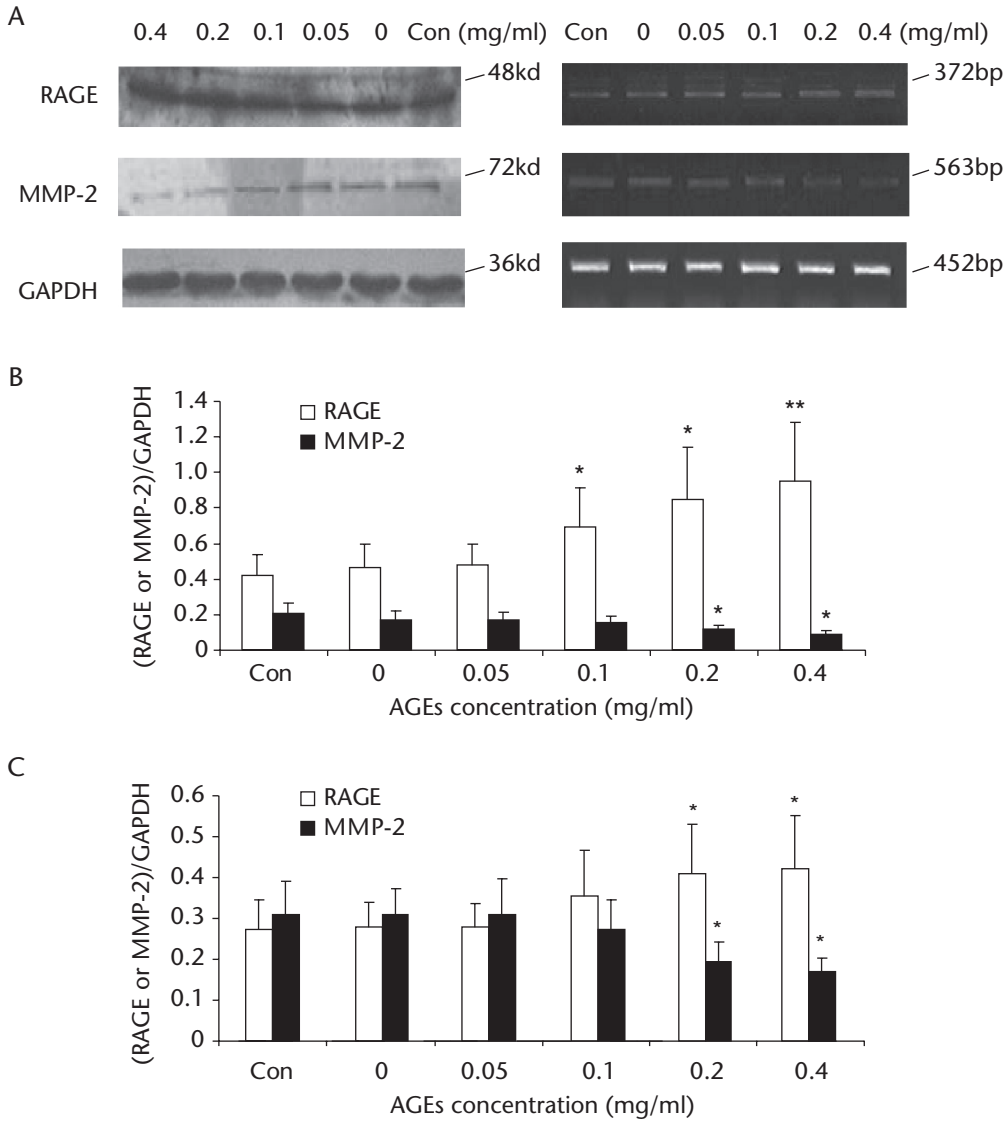

FIGURE 1: Protein and gene expression of the receptor for advanced glycation end products (RAGE) and matrix metalloproteinase-2 (MMP-2) in human umbilical vein endothelial cells after incubation for 24 h with various concentrations of advanced glycation end products (AGEs) or with 0.4 mg/ml negative control of AGEs (Con). (A) Western blot and reverse transcription-polymerase chain reaction (RT-PCR); (B) density analysis of Western blot bands; (C) density analysis of RT-PCR bands. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus 0 mg/ml; $n = 3$

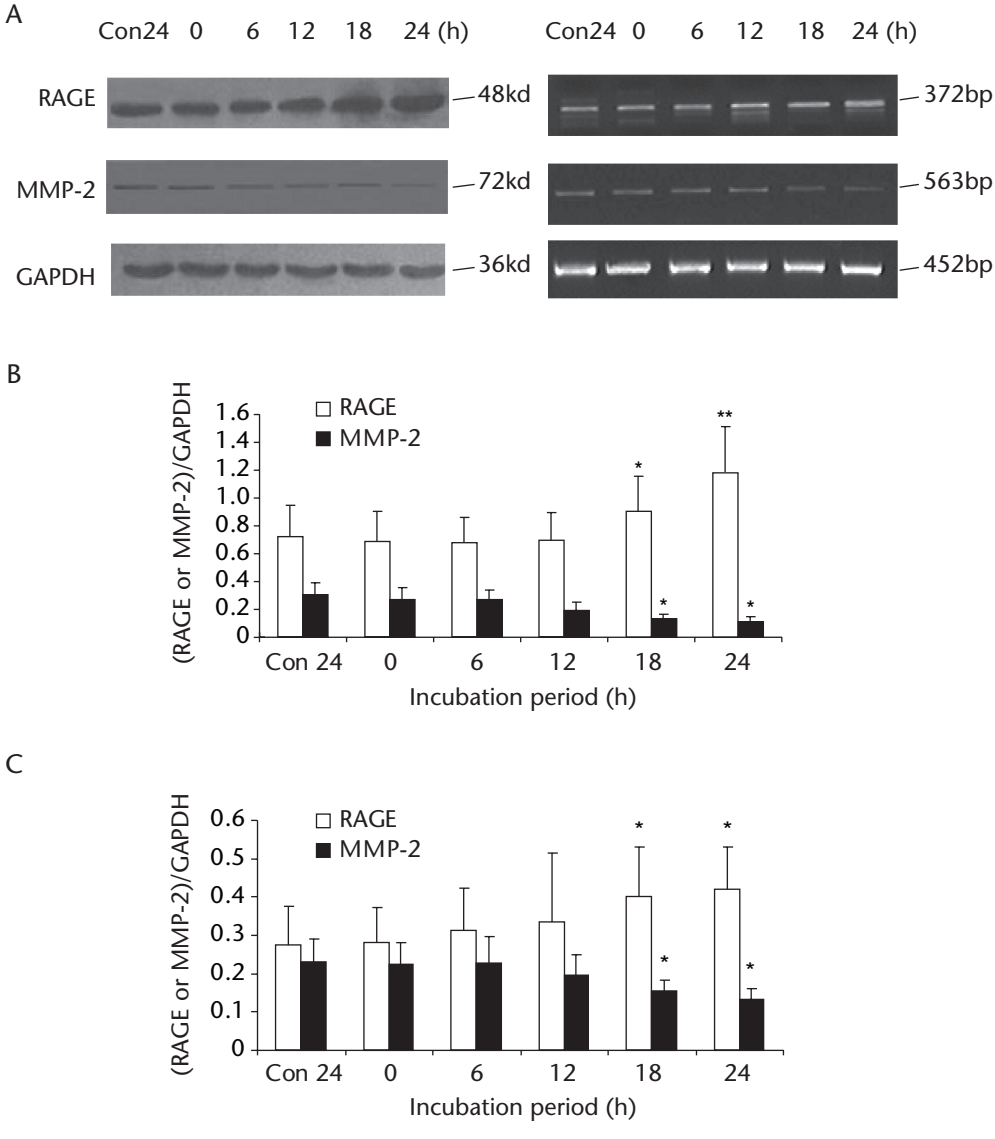

FIGURE 2: Protein and gene expression of receptor for advanced glycation end products (RAGE) and matrix metalloproteinase-2 (MMP-2) in human umbilical vein endothelial cells after incubation with 0.2 mg/ml advanced glycation end products (AGEs) for various time periods or with 0 mg/ml AGEs for 24 h (Con 24). (A) Western blot and reverse transcription-polymerase chain reaction (RT-PCR); (B) density analysis of Western blot bands; (C) density analysis of RT-PCR bands. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus 0 h; $n = 3$

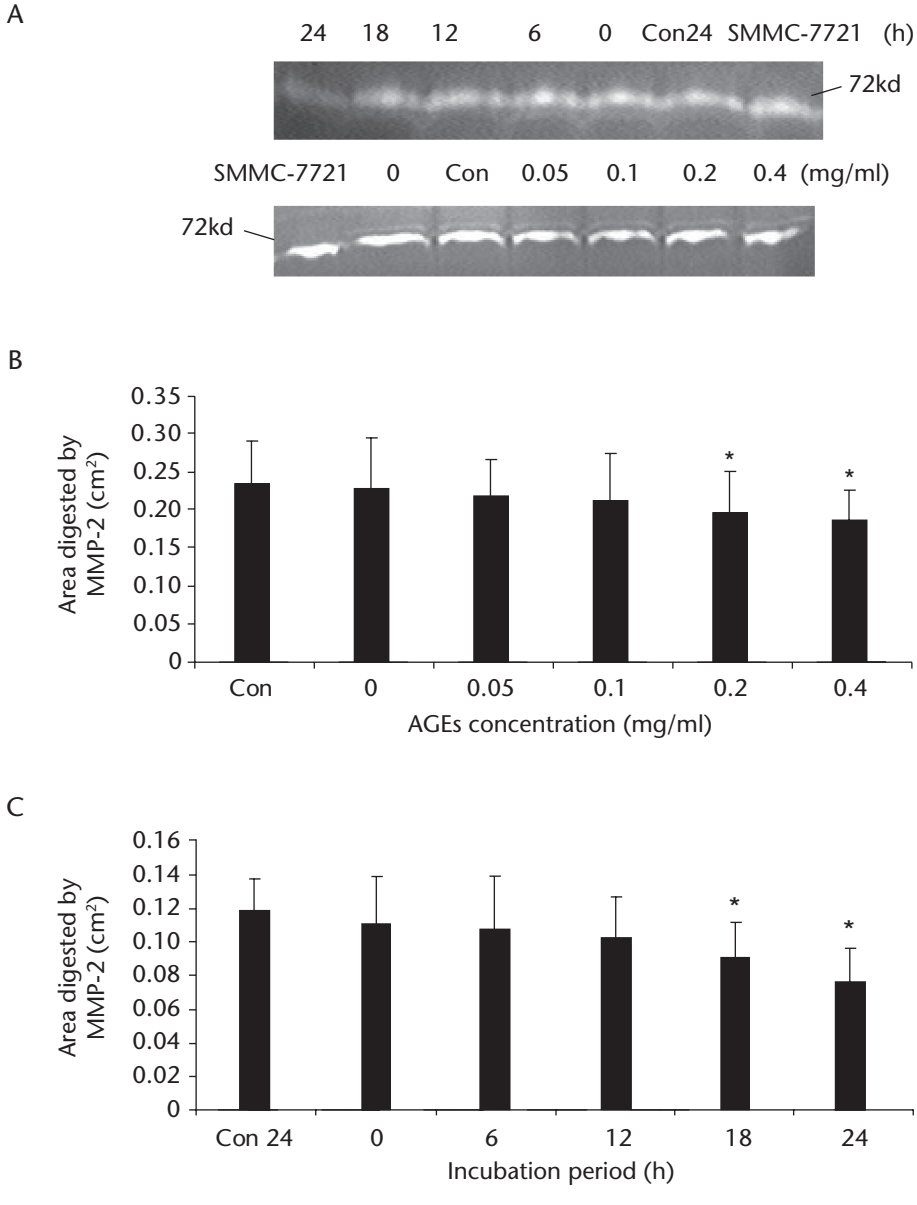

FIGURE 3: Matrix metalloproteinase-2 (MMP-2) activity in conditioned medium of human umbilical vein endothelial cells after incubation with various concentrations of AGEs for 24 h or with 0.4 mg/ml negative control of AGEs (Con), or with 0.2 mg/ml AGEs for various time periods or with 0 mg/ml AGEs for 24 h (Con 24). (A) Gelatin zymography; (B) Area digested by MMP-2 at differing concentrations of AGEs; (C) Area digested by MMP-2 after differing time periods. $^{*}P < 0.05$ versus 0 mg/ml or versus 0 h; $n = 3$

protein and gene expression were time and concentration dependent (Figs 1 and 2). Incubation with AGEs at concentrations of ≥ 0.2 mg/ml for 24 h significantly inhibited MMP-2 expression but significantly increased RAGE expression compared with controls. Incubation with 0.2 mg/ml AGEs for ≥ 18 h significantly inhibited MMP-2 but significantly increased RAGE expression compared with controls.

EFFECTS ON MMP-2 ACTIVITY IN CONDITIONED MEDIUM

The effects of AGEs on MMP-2 activity in the conditioned medium were also time and concentration dependent (Fig. 3). Both latent and active MMP-2 were found, with latent MMP-2 being the main form in HUVECs.

Discussion

An increasing number of clinical trials suggest that patients with diabetes suffer more serious coronary and peripheral artery disease and have increased morbidity and mortality.[9] The serum concentration of AGEs is almost 10 times higher in diabetic patients than in non-diabetic subjects.[1] There is growing evidence supporting the involvement of AGEs and MMPs in atherosclerosis, retinopathy, nephropathy and other diabetic complications.[1–4,6] It has been reported that AGEs influence the expression and activity of MMPs in cells such as macrophages and choroidal endothelial cells;[3,6] however, the effects of AGEs on MMP expression and activity in vascular endothelial cells has not been investigated.

In the present study, AGEs increased RAGE protein and gene expression but decreased MMP-2 protein and gene expression in HUVECs, and inhibited MMP-2 activity in conditioned medium in a time- and concentration-dependent manner. Since RAGE is a signal transduction receptor for AGEs on the cell surface,[10] these results suggest that the effects of AGEs on MMP-2 may be mediated through RAGE. As the serum concentration of AGEs is approximately 0.2 mg/ml in diabetic patients,[1] the changes seen in this study may occur in diabetes.

Matrix metalloproteinases are very important in maintaining ECM homeostasis and participate in many physiological and pathological processes. Disturbed ECM remodelling is involved in the pathogenesis of atherosclerosis and other vascular disorders.[11–13] Decreased secretion and activity of MMP-2 in endothelial cells may disturb the turnover of subendothelial ECM,[5] resulting in accumulation of abnormal matrix, thickening and hardening of the basement membrane and endothelial dysfunction, thus influencing angiogenesis or contributing to vascular complications. The present results suggest that AGEs may alter endothelial function by decreasing MMP-2 expression and activity in endothelial cells. The effects of AGEs on MMPs in the endothelium of diabetic patients need further study.

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Conflicts of interest

No conflicts of interest were declared in relation to this article.

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