Selective Increase in Decorin Core mRNA Level in Cultured Vascular Smooth Muscle Cells after Exposure to Advanced Glycation Endproducts

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Although advanced glycation endproducts (AGEs) have been proved to be involved in the pathogenesis of vascular complications, such as atherosclerosis in diabetic patients, little is known about the functional damage to vascular cells caused by AGEs. In early and late atherosclerosis, excess proteoglycans (PGs) derived from vascular smooth muscle and endothelial cells deposit in the subendothelial extracellular matrix of the vascular wall. To address the question whether AGEs affect the synthesis of PGs in vascular cells or not, the effect of AGEs on steady-state levels of PG core mRNAs in cultured human aortic smooth muscle and endothelial cells was investigated. AGEs were prepared by incubation of bovine serum albumin with glucose and the mRNAs coding for the core proteins of heparan sulfate PGs (perlecan and syndecan-1) and chondroitin/dermatan sulfate PGs (versican, biglycan and decorin) were determined by quantitative reverse transcription-polymerase chain reactions. In vascular smooth muscle cells, the steady-state level of mRNAs coding for perlecan, syndecan-1, versican and biglycan core proteins was unchanged whereas that of mRNA coding for decorin core protein was increased by AGEs in a dose- and time-dependent manner. On the other hand, in vascular endothelial cells, mRNAs coding for perlecan, syndecan-1 and biglycan were observed, although their steady-state levels were unaffected by AGEs. The present data suggest that AGEs may be involved in the accumulation of decorin derived from vascular smooth muscle cells in the atherosclerotic vascular wall of diabetic patients.

Key words —— advanced glycation endproduct, vascular, proteoglycan, decorin, diabetes

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INTRODUCTION

Proteoglycans (PGs) are macromolecules composed of a core protein and one or more glycosaminoglycan (GAG) side chain(s) as a common feature. PGs synthesized by vascular endothelial and smooth muscle cells play an important role in the regulation of arterial properties, such as permeability, lipid metabolism, hemostasis, thrombosis and extracellular matrix assembly.[1–4] In early and late atherosclerosis, however, excess PGs accumulate in the intima and are involved in pathogenesis of the lesion.[2,5] For example, low density lipoprotein (LDL) is bound to GAG chains such as chondroitin/dermatan sulfate[6] and the GAG-LDL complexes in atherosclerotic plaques[7] enhance the accumulation of LDL by macrophages.[8] In addition, diabetes-induced glycation of LDL increases its PG binding properties.[9]

One of the conditions associated with the rapid onset of atherosclerosis is diabetes.[10] The PG metabolism in vascular endothelial and/or smooth muscle cells may be relevant to atherosclerosis in diabetes because changes in the PG component are associated with the vascular lesion.[11] In fact, dermatan sulfate, a GAG constituent with very high affinity for LDL, is significantly increased in coronary arteries in alloxan-diabetic dogs.[12] However, the mechanism for the changes in the PG component of atherosclerosis in diabetic patients is unclear.

Reducing sugars, such as glucose and fructose, can react non-enzymatically with amino groups of proteins to form reversible Schiff bases and then Amadori products. These early glycosylation products undergo further reactions and rearrangements to become irreversible cross-

linked and fluorescent protein derivatives termed advanced glycation endproducts (AGEs).[13] Hyperglycemia-induced accumulation of AGEs in various tissues has been implicated in the development of diabetic complications.[14,15] Since several cell types including endothelial cells have receptors for AGEs,[16] we hypothesized that PG synthesis in vascular endothelial and/or smooth muscle cells may be accelerated by AGEs.

Although the expression of PGs differs between vascular smooth muscle cells and endothelial cells,[17] both cell types are capable of synthesizing heparan sulfate PGs, including perlecan and syndecan-1, and chondroitin/dermatan sulfate PGs, including versican, biglycan and decorin. In our present study, we investigated the steady-state level of mRNAs coding for core proteins of these PGs in cultured vascular smooth muscle and endothelial cells after exposure to AGEs. Our experiments show that only decorin core mRNA in vascular smooth muscle cells is increased by AGEs.

MATERIALS AND METHODS

Materials —— Vascular smooth muscle and endothelial cells from human aorta were purchased from Krabo (Oosaka, Japan). Fetal bovine serum was from Summit (Ft. Collins, CO, U.S.A.). RPMI 1640 medium was obtained from Nissui Pharmaceutical (Tokyo, Japan). Tissue culture dishes and plates were from Iwaki (Tokyo, Japan). Bovine serum albumin fraction V (fatty acid-free, low endotoxin) was purchased from Boehringer Mannheim GmbH (Germany). Heparin-Sepharose CL-4B was from Pharmacia LKB (Uppsala, Sweden). Reverse transcriptase was obtained from Takara (Kyoto, Japan). Ampli Taq DNA polymerase was from Perkin Elmer (Foster, CA, U.S.A.). Transfer membranes (Hybond N^+) were purchased from Amersham (Little Chalfont, U.K.). Other reagents were from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of AGEs —— BSA was incubated with 50 mM glucose in phosphate-buffered saline (PBS) at 37°C for 6 weeks and unincorporated sugar was removed by dialysis against PBS. AGEs were purified by a heparin-Sepharose CL-4B column. Separation of AGEs from non-glycated BSA was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The concentration of AGEs was determined by the method of Bradford.[18]

Cell Culture —— Vascular smooth muscle and endothelial cells were each cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humid atmosphere of 5% CO_2 in air until confluent. They were then incubated at 37°C for 4, 8, 24 or 48 h in the fresh medium in the presence or absence of AGEs at 50, 100 or 200 μg/ml.

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) —— Poly(A)$^+$RNA was isolated from the cells and analyzed by quantitative RT-PCR as previously described.[19,20] PCR was conducted under quantitative conditions, which were determined by plotting signal intensities as a function of the template amount and cycle number. Oligonucleotide primers were designed on the basis of sequences of human cDNAs. The sequences of the upstream and downstream primers were as follows: 5'-ATGAGTACATGCTGGCTGAC-3' and 5'-CTGATACCCAGGACTGGCTC-3', which corresponded to nucleotides (nt) 247—266 and 786—805 of perlecan core cDNA; 5'-TGCCCCCTGAAGATCAAGAT-3' and 5'-CAGGGGTTGAGGTCTCATGG-3', which corresponded to nt 291—310 and 688—707 of syndecan-1 core cDNA; 5'-AAAGATTTGAAAGAGACTAC-3' and 5'-TCAGTAGCATTTGCACACTC-3', which corresponded to nt 492—511 and 1488—1507 of versican core cDNA; 5'-AAACTGCCCAGGAGT-3' and 5'-GGCTGACTAGTTGGC-3', which corresponded to nt 514—528 and 598—612 of biglycan core cDNA; 5'-CCATTCAACTCGGAAACTAT-3' and 5'-ATAGCCTGTATTGAATTCAT-3', which corresponded to nt 291—310 and 765—784 of decorin core cDNA. The PCR amplified fragments were electrophoresed on 2% agarose gel containing 0.1 μg/ml etidium bromide. The size of the PCR products for perlecan, syndecan-1, versican, biglycan and decorin core proteins was 559, 417, 1016, 99 and 494 base pairs, respectively.

RESULTS

Figure 1 shows the steady-state levels of mRNAs coding for PG core proteins in vascular smooth muscle cells after exposure to AGEs. In this cell type, mRNAs coding for all tested PG core proteins (perlecan, syndecan-1, versican, biglycan and decorin) were detected. When the cells were exposed to AGEs at 100 μg/ml, only decorin core mRNA was increased in a time-dependent manner; mRNAs coding for other PG core proteins were unchanged by AGEs.

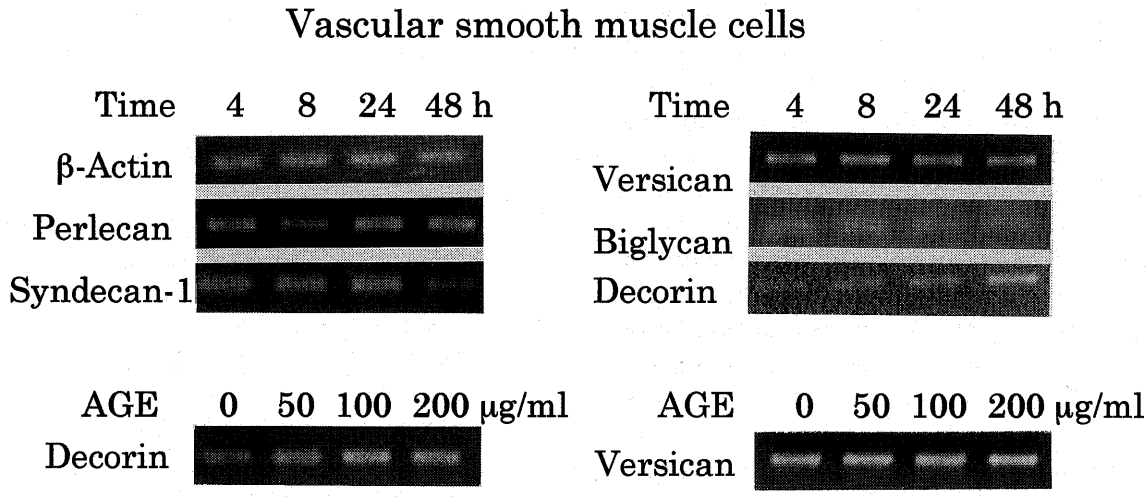

Fig. 1. Quantitative RT-PCR Analysis of PG Core Protein mRNAs in Cultured Vascular Smooth Muscle Cells after Exposure to AGEs

Human aortic smooth muscle cells were incubated at 37°C for 4, 8, 24 or 48 h with AGEs at 100 μg/ml or for 24 h with AGEs at 50, 100 or 200 μg/ml.

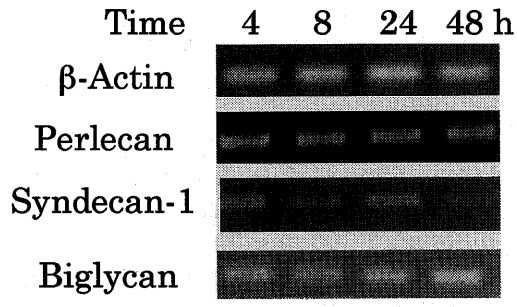

Fig. 2. Quantitative RT-PCR Analysis of PG Core Protein mRNAs in Cultured Vascular Endothelial Cells after Exposure to AGEs

Human aortic endothelial cells were incubated at 37°C for 4, 8, 24 or 48 h with AGEs at 100 μg/ml.

Although the amount of versican core mRNA was not changed by AGEs at 200 μg/ml and below after a 24-h exposure, decorin core mRNA was increased in a dose-dependent manner.

On the other hand, although we failed to detect mRNAs coding for versican and decorin core proteins, mRNAs coding for perlecan, syndecan-1 and biglycan core proteins could be determined in vascular endothelial cells. As shown in Fig. 2, the amount of detected PG core mRNAs was unchanged by AGEs at 100 μg/ml up to 48 h.

DISCUSSION

The regulation of the synthesis of individual PGs differs among different cell types. For example, transforming growth factor beta (TGF-β), a cytokine that regulates PG synthesis stimulates the synthesis of versican and biglycan but does not affect decorin in human fibroblasts.[21,22] However, the cytokine causes an increase in the synthesis of both biglycan and decorin in rat myocardial fibroblasts.[23] In vascular smooth muscle cells, TGF-β promotes the synthesis of versican[24] and biglycan[25] but does not affect the synthesis of decorin core protein, although the length of the decorin GAG chains is extended.[25] Moreover, in endothelial cells, the synthesis of biglycan core protein is promoted by TGF-β, regardless of the cell density, and that of perlecan is also promoted by the cytokine only when the cell density is high.[26] In the present study, it was demonstrated that AGEs increase the steady-state level of decorin core mRNA in vascular smooth muscle cells. In other words that vascular smooth muscle cells are a particular cell type whose decorin core synthesis responds to AGEs.

Biglycan and decorin are closely related, small, leucine-rich chondroitin/dermatan sulfate PGs[27] and are found in the arterial wall.[28] The core proteins of both biglycan and decorin bind TGF-β and sequester the cytokine from the cell surface receptor.[29] It has been proposed that up-regulation of small leucine-rich PG synthesis may limit TGF-β activity.[25] Thus, it is suggested

that AGEs may reduce TGF-β activity by induction of decorin synthesis in vascular smooth muscle cells. On the other hand, LDL is bound to chondroitin/dermatan sulfate chains in atherosclerotic plaques,[6] and the formation of GAGs-LDL complexes enhances the accumulation of low density lipoprotein by macrophages.[8] Since chondroitin/dermatan sulfate chains of decorin, as well as versican and biglycan, bind LDL, AGEs appear to contribute to the accumulation of LDL in the vascular wall through promotion of decorin synthesis in vascular smooth muscle cells.

Endothelial cells are involved in the regulation of the blood coagulation-fibrinolytic system. The cells synthesize tissue factor which activates extrinsic coagulation, thrombomodulin, which converts thrombin activity from coagulation to anticoagulation,[30] tissue plasminogen activator, which converts plasminogen to plasmin on the fibrin clot,[31] and plasminogen activator inhibitor-1, which inhibits tissue plasminogen activator.[32] It has been reported that AGEs suppress the expression of thrombomodulin and tissue plasminogen activator but enhance that of tissue factor and plasminogen activator inhibitor-1,[33] suggesting that AGEs induce a procoagulant state in the blood. Since there is a strong relationship between atherosclerosis and the procoagulant state of blood, an assumption can be made that the pathogenesis of complicated atherosclerosis in diabetes includes functional damage of the endothelial cells. The present data suggest that AGEs can cause functional damage to vascular smooth muscle cells as well as to endothelial cells *e.g.* by induction of decorin synthesis.

Although the present study shows that the steady-state level of decorin core mRNA is increased by AGEs in vascular smooth muscle cells, the following questions remain to be answered: 1) What are the structural changes of PGs such as elongation of GAG side chains induced by AGEs? 2) How is expression of decorin core protein in vascular smooth muscle cells induced by AGEs? 3) What is the involvement of receptors for AGEs in the increase in decorin mRNA in vascular smooth muscle cells? 4) What are the effects of AGEs on vascular smooth muscle cell functions including proliferation, migration and collagen synthesis? However, the present study is the first evidence showing possibile abnormal metabolism of PG synthesis by AGEs in vascular smooth muscle cells.

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