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High glucose enhance expression of matrix metalloproteinase-2 in smooth muscle cells¹

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KEY WORDS glucose; matrix metalloproteinases; gelatinases; diabetes mellitus; arteriosclerosis; vascular smooth muscle

ABSTRACT

AIM: To investigate the effects of high glucose on expression of matrix metalloproteinase-2 (MMP-2) in rat aortic smooth muscle cells and the influence of matrix remodeling on atherogenesis in diabetic patients. **METHODS:** The smooth muscle cells were cultured from the thoracic aorta of Sprague-Dawley (SD) rat. MMP-2 mRNA was determined by reverse transcriptase-polymerase chain reaction (RT-PCR), MMP-2 protein was measured by Western blotting, and MMP-2 activity in conditioned medium was observed by zymography. **RESULTS:** In comparison with the control, there was no difference in the expression of MMP-2 when glucose concentration was 1 g/L, whereas MMP-2 activity in smooth muscle cells was significantly increased by the glucose 5 g/L ($P < 0.01$). **CONCLUSION:** High glucose enhanced the expression and activity of MMP-2 in smooth muscle cells, which may provide an explanation for the phenomenon that diabetes patients are prone to have atherosclerotic lesions.

INTRODUCTION

Diabetes is a common disease, and cardiovascular complications are the leading cause of morbidity and mortality. The blood glucose of high concentration is the key factor inducing those cardiovascular complications^[1]. High glucose concentration can induce production of many cytokines and initiate all kinds of pathophysiological process. The proliferation and migration of vascular smooth muscle cells are important steps in formation and development of atherosclerotic lesions, and the degradation and remodeling of vascular base-

ment membrane play a crucial role in the process^[2-3]. The matrix metalloproteinases (MMP) are a family of zinc-dependent enzymes which have a high affinity for extracellular matrix components and therefore play a fundamental role in matrix remodeling processes. The remodeling of basement membrane is especially involved in MMP-2 (ie, gelatinase A). MMP-2 can degrade gelatin, several kinds of collagens, and components of vascular basement membrane, hence it can affect the remodeling of vascular extracellular matrix^[4]. Many cytokines and atherogenesis factors can increase the production of MMP-2. In the present study, we observed the change of expression of MMP-2 in cultured rat aortic smooth muscle cells exposed to high glucose concentration by which diabetic patients have the tendency to suffer from atherosclerosis and plaque rupture.

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MATERIALS AND METHODS

Reagents DMEM low glucose medium (Gibco), TriZOL (Gibco), glucose (Shanghai Chemical Reagents Factory), Mu-MLV reverse transcriptase (Promega), *Taq* DNA polymerase (Promega), goat-anti-rat MMP-2 polyclonal antibody (Santa Cruz), rabbit-anti-goat-HRP (SABC), ECL chemiluminescence reaction detection reagents (ECL Western blotting, Amersham), gelatin (Sigma)

Cell culture Aortic smooth muscle cells were cultured with thoracic aorta excised from Sprague-Dawley rats (Grade II, Shanghai BK Company) by substrate-attached explants and incubated in Dulbecco's modified Eagle's medium (DMEM). Cells available for experiments were 6-8 passages. Cells were grown in control (glucose 0 g/L), glucose 1 g/L or glucose 5 g/L medium for 24 h or 48 h.

Semiquantitative reverse transcriptase (RT)-PCR^[5] Total RNA was extracted with TriZOL. The first-strand cDNA was synthesized from the total RNA using Mu-MLV reverse transcriptase (Promega) and oligo (DT)18 primers (Sangon, Shanghai) according to the standard method. The reverse transcription reaction was performed at 42 °C for 1 h, at the end of reverse transcription, the mixture was heated at 95 °C for 5 min and immediately cooled on ice for 5 min. The second-strand DNA synthesis and 30-cycle amplification were performed. Direct and reverse oligo primers for MMP-2 (5' GCT GAT ACT GAC ACT GGT ACT G 3' for sense and 5' CAA TCT TTT CTG GGA GCT C 3' for antisense) and β -actin (5' GGT ATG GGT CAG AAG GAC TCC 3' for sense and 5' TGA TCT TCA TGG TGC TAG GAG CC 3' for antisense) were constructed to amplify the full coding sequences according to the published sequences. Amplification was performed for 30 cycles of denaturation (60 s at 94 °C), annealing (60 s at 60 °C), and elongation (90 s at 72 °C). PCR products were electrophoresed through a 1.5 % agarose gel and visualized by ethidium bromide staining under UV light. The relative intensities of the bands were quantified by densitometric analysis.

Western blot^[6] The cells were homogenized in PBSTDS lysis buffer containing 10×PBS (10 %), Triton X-100 1 %, sodium deoxycholate 5 g/L, SDS 1 g/L, aprotinin 0.5 mg/L, PMSF 1 mmol/L, and edetic acid 1 mmol/L at 4 °C and harvested by a cell scraper. The protein concentrations were determined by BCA kit. Protein samples (20 μ g) were separated on 10 %

SDS polyacrylamide gels and electrophoretically transferred to an Hybond Nitrocellulose (NC) membrane (Amersham Life Science, Inc) by a transfer system (BIO-RAD). Effect of transferring was confirmed by staining the NC membrane with Ponceau S solution. The nonspecific binding on the NC membrane was blocked by incubation with 10 % nonfat dry milk and 0.1 % Tween-20 in TBS (TBST) on shaker at room temperature for 2 h. The membrane was incubated with goat anti-rat MMP-2 polyclonal antibody (Santa Cruz) at 1:200 dilution in TBST at 37 °C for 2 h. The membrane was washed 3 times with TBST for 15 min and incubated at room temperature with a rabbit anti-goat horseradish peroxidase-conjugated secondary antibody at 1:300 dilution for 1 h. The membrane was washed 3 times with wash buffer and incubated at room temperature in chemiluminescence reaction detection reagents (ECL, Amersham) for 1 min. The membrane was then exposed to autoradiography film (HyperfilmECL, Amersham). The relative intensities of the bands were quantified by densitometric analysis.

Gelatin zymography^[7] Gelatinolytic activities of conditioned media were analyzed by vertical electrophoresis in the presence of 10 % SDS-polyacrylamide gels containing 1 g/L gelatin. After electrophoresis, the gels were rinsed in 2.5 % TritonX-100 for 30 min and incubated at 37 °C in Tris-HCl 50 mmol/L (pH 7.8), CaCl₂ solution 10 mmol/L for 24 h. Then gels were stained with Coomassie brilliant blue R-250, and the cleaved bands were quantified by densitometric analysis.

Statistical analysis All numerical data were expressed as mean \pm SD. Statistical analyses were performed by Student's *t* test for experiments consisting of two groups only and by ANOVA and Dunnett *t* test for experiments consisting of more than two groups. Statistical significance was defined as $P < 0.05$.

RESULTS

Effects of high glucose on MMP-2 mRNA in ASMC In comparison with control, glucose 1 g/L did not change the MMP-2 mRNA level in ASMC. The expression of MMP-2 mRNA was increased when glucose concentration reached to 5 g/L ($P < 0.01$), and when the incubation time was extended to 48 h, MMP-2 mRNA expression had no difference from that for 24 h at glucose 5 g/L (Fig 1, 2).

High glucose increased expression of MMP-2 protein in ASMC MMP-2 protein expression in ASMC was increased by glucose 5 g/L, but not by glucose 1

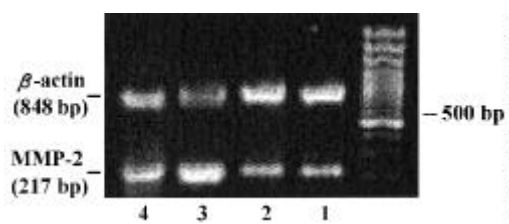


Fig 1. MMP-2 mRNA expression in ASMC determined by RT-PCR. 1) Control, 24 h; 2) Glucose 1g/L, 24 h; 3) Glucose 5 g/L, 24 h; 4) Glucose 5 g/L, 48 h.

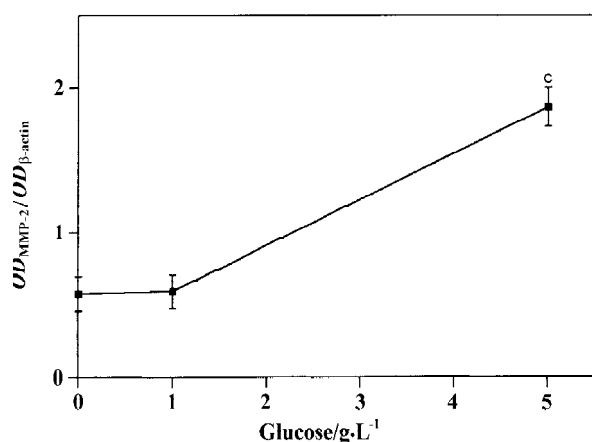


Fig 2. Effect of different glucose concentration on MMP-2 mRNA expression in ASMC for 24 h. $n=3$. Mean \pm SD. ^c $P<0.01$ vs control. OD: optical density.

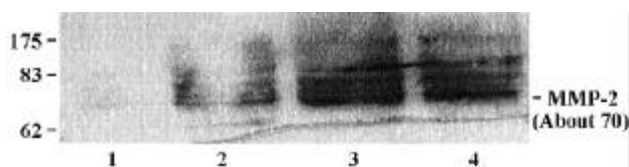


Fig 3. Western blot analysis of MMP-2 protein production in ASMC. 1) Control, 24 h; 2) Glucose 1 g/L, 24 h; 3) Glucose 5 g/L, 24 h; 4) Glucose 5 g/L, 48 h.

g/L ($P<0.01$) (Fig 3, 4). When cells were incubated for 48 h, the MMP-2 protein expression was similar to that for 24 h.

High glucose enhanced the MMP-2 activity
MMP-2 protein in conditioned media was measured by gelatin zymography, the conditioned medium containing glucose 5 g/L showed a stronger gelatinolytic activity than that of control or glucose 1 g/L ($P<0.01$) (Fig 5,6). The conditioned medium which contained 5 g/L glucose and was incubated for 48 h manifested the same gelatinolytic activity with that for 24 h.

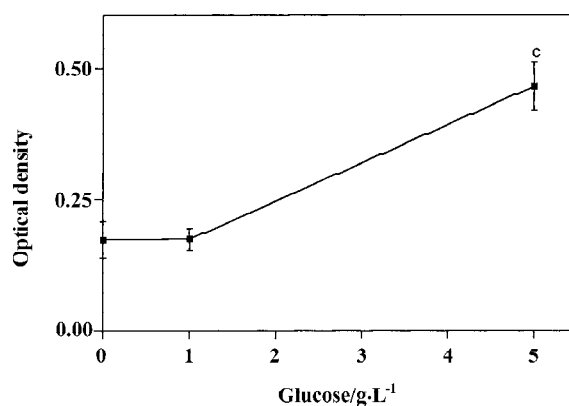


Fig 4. Effect of different glucose concentration on MMP-2 protein expression in ASMC for 24 h. $n=3$. Mean \pm SD. ^c $P<0.01$ vs control.

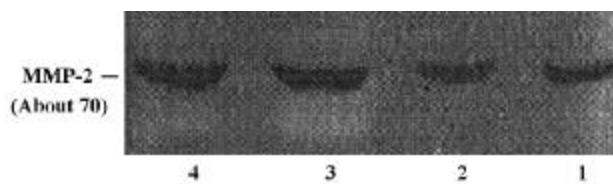


Fig 5. Gelatinolytic activity of conditioned media containing different glucose concentration. 1) Control, 24 h; 2) Glucose 1 g/L, 24 h; 3) Glucose 5 g/L, 24 h; 4) Glucose 5 g/L, 48 h.

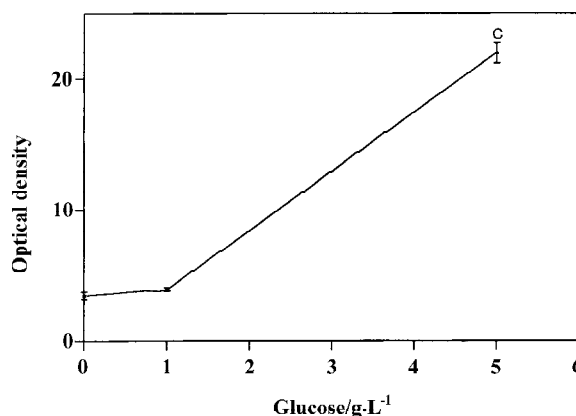


Fig 6. Effect of different glucose concentration on gelatinolytic activities of conditioned media. $n=3$. Mean \pm SD. ^c $P<0.01$ vs control.

DISCUSSION

Extracellular matrix remodeling exists in many cardiovascular diseases, such as coronary heart disease, cardiomyopathy, and aortic aneurysm, *etc.* The production, degradation, and remodeling of matrix af-

fects the initiation and development of these diseases. Therefore, the MMP family which can impact the balance between synthesis and degradation of collagen matrix has important influence on the remodeling of vascular extracellular matrix^[8]. MMP family consists of interstitial collagenases, gelatinases, stromelysins, and membrane type-collagenases, *etc*^[9]. Gelatinase A, ie MMP-2, plays an important role in the proliferation and migration of vascular smooth muscle cells, so we can understand pathogenesis of many cardiovascular diseases by investigating the expression and role of MMP-2. Li and colleague^[10] have demonstrated that the expression of MMP-2 was increased in human aortic atherosclerotic lesions and MMP-2 may have a role in the formation and progression of the atherosclerotic lesion and may be involved in clinical complications of atherosclerosis, such as fissure and rupture, leading to thrombosis. Several kinds of cytokines, such as TNF- α , IL-1 β ^[11], and thrombin^[12] can promote activation of matrix metalloproteinase-2 in cultured vascular smooth muscle cells. In the case of complicated atherosclerotic plaques, episodes of intraplaque hemorrhage or plaque disruption with thrombosis may lead to plaque instability by increasing local matrix-degrading activity.

High blood glucose is the basal manifestation of diabetes, high glucose can not only cause the typical symptoms but also induce and increase the production of many cytokines so that it may initiate series of pathophysiological processes. High glucose can promote the proliferation of smooth muscle cells and inhibit the anti-proliferative effect of estrogen^[14]. Furthermore, high glucose can augment osteopontin expression in smooth muscle cells^[15]. Our study observed the influence of high glucose on MMP-2 expression in cultured rat aortic smooth muscle cells and the MMP-2 gelatinolytic activity in conditioned medium. The results showed that MMP-2 was expressed constitutively in smooth muscle cells when glucose concentration was 0 or 1 g/L, but the expression was increased when glucose concentration was 5 g/L and incubation time was 24 h ($P < 0.01$), and when incubation time was extended to 48 h, the expression was not different from that for 24 h. Although the influence of high blood glucose is a chronic and long-term process, we had cultured the cells in medium of high glucose (5 g/L) for 24-48 h, which should reflect something about the pathogenesis of diabetic complications. From the above study, we can also understand lowering-blood glucose therapy not only can attenuate symptoms of diabetes mellitus, but

also decrease the incidence of cardiovascular disease and long term mortality.

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