Journal of Cellular Biochemistry 101:442–450 (2007)

Opposite Effects of High Glucose on MMP-2 and TIMP-2 in Human Endothelial Cells

Feng M. Ho,[1,2]* Shing H. Liu,[3] Wan W. Lin,[4] and Chiau S. Liau[5]

[1]Department of Internal Medicine, Tao-Yuan General Hospital, Taiwan
[2]Department of Health, the Executive Yuan, Taiwan
[3]Institute of Toxicology, National Taiwan University, Taiwan
[4]Institute of Pharmacology, National Taiwan University, Taiwan
[5]Department of Internal Medicine, National Taiwan University Hospital, Taiwan

Abstract Diabetes mellitus (DM) is a major risk factor for atherosclerosis and causes multiple cardiovascular complications. Although high glucose can induce matrix metalloproteinases (MMPs), its inhibitors and cell apoptosis, little is known about the roles of MMPs in regulating cell apoptosis in response to high glucose. To address this issue, we elucidated the relationship between MMPs, its inhibitors and cell apoptosis in human umbilical vein endothelial cells (HUVECs). HUVECs were treated with medium containing 5.5 mM or 33 mM of glucose in the presence or the absence of ascorbic acid and MMP inhibitors (GM6001 and endogenous tissue inhibitors of MMPs, TIMP-1, and TIMP-2). For detection of cell apoptosis, the cell death detection ELISA assay was used. The results revealed that high glucose-induced apoptosis could be suppressed by ascorbic acid, GM6001 and TIMP-2, but not by TIMP-1. The activities of MMP-2, MMP-9 and its inhibitors, TIMP-1, TIMP-2 after high glucose treatment, were also detected by ELISA method. We found that the activated form of MMP-2, but not MMP-9, was increased, while the level of TIMP-2, but not TIMP-1, was decreased. In Western blot and RT-PCR analysis, the expression of TIMP-2, but not TIMP-1, after high glucose treatment was downregulated, whereas the levels of MMP-2 and -9 proteins and mRNA were not changed. The present study indicated that oxidative stress induced by high glucose might be involved in the opposite effects on MMP-2 activation and TIMP-2 downregulation. This reactive oxygen species (ROS)-dependent MMP-2 activation in turn mediates high glucose-induced cell apoptosis in HUVECs. J. Cell. Biochem. 101: 442–450, 2007.

Key words: matrix metalloproteinase; high glucose; apoptosis; human endothelial cell

Diabetes mellitus (DM) is one of the major risk factor in patients with cardiovascular disease [Kannel and Mcgee, 1979; Rudermam and Haudenchild, 1984]. Hyperglycemia could be a contributing factor to accelerate atherosclerosis and develop the vascular complications [The Diabetes Control and Complications Trial Research Group, 1993]. Evidence showed that elevated glucose concentrations can induce dysfunction of several intracellular signal transduction cascades, which leads to an upset in the balance between extracellular matrix (ECM) synthesis and degradation [Fisher et al., 1991; Puliese et al., 1994]. Localized ECM breakdown plays a major role in the pathogenesis of atherosclerosis [Galis et al., 1995]. However, the detail molecular mechanisms are not yet clear.

Matrix metalloproteinases (MMPs) are members of family of Zn^{+}- and Ca^{+}-dependent endopeptidases, which are secreted by many types of cells as proleolytic enzymes. On activation by proteolytic cleavage, activated enzymes are capable of degrading many ECM components. MMPs are produced and generally secreted in a latent form that requires stepwise activation through interactions with a variety of proteins, including membrane-type (MT)-MMPs and adhesion molecules [Borden and Heller, 1997; Nagase, 1997]. Increased expression and activity of MMPs have been identified in various pathological processes,

*Correspondence to: Feng M. Ho, MD, PhD, Department of Internal Medicine, Taoyuan General Hospital, Department of Health, the Executive Yuan. No. 1492, Chung Shan Road, Taoyuan City, Taiwan.
E-mail: heart@mail.tygh.gov.tw

Received 4 April 2006; Accepted 5 October 2006
DOI 10.1002/jcb.21192

such as general inflammation, atherosclerosis, and cardiovascular disease [Galis et al., 1994; Creemers et al., 2001; Komorowski et al., 2002]. Moreover, evidence demonstrated that MMPs, especially MMP-2 and MMP-9 are actively synthesized in atheromatous plaque and are particularly prevalent in rupture-prone shoulder regions [Galis et al., 1994], which may lead to acute coronary syndrome. Both MMP-2 and MMP-9 can be activated by reactive oxygen species (ROS), and their expression seems to be regulated by oxidative stress [Rajagopalan et al., 1996]. However, the potentially deleterious nature of MMPs is tightly regulated at different levels, including transcriptional and post-transcriptional levels. Post-transcriptional regulation involves binding to specific endogenous tissue inhibitors of MMPs (TIMPs), which may prevent proteolytic cleavage of the proenzyme, as well as function of the active enzyme [Gomez et al., 1997]. It is currently accepted that the balance between MMP and TIMP activity in a particular environment may determine their net degrading potential [Gomez et al., 1997; Nagase and Woessner, 1999]. Evidence also demonstrated that high glucose could induce discordant regulation of MMP/TIMP system, which may be influential the processing atherosclerosis [Death et al., 2003]. In our previous study, apoptosis induced by high glucose in human endothelial cell was also observed [Ho et al., 2000]. Nonetheless, the relationships among hyperglycemia, apoptosis, and MMP/TIMP gene regulation and activities have not been investigated. Here, we try to study the mechanism and contribution of regulating MMP/TIMP in endothelial cell apoptosis after exposure to high glucose (33 mM).

MATERIALS AND METHODS

Cell Culture and Treatment

Human umbilical vein endothelial cells (HUVECs) were cultured as previously described [Ho et al., 2000]. Cells were seeded at a density of 1×10^5 per 75-cm^2-flask in medium 199 (Gibco, Grand Island, NY), supplemented with 20 mM HEPES, 100 μg/ml endothelial cell growth substance (Collaborative Research, Inc., Bedford, MA), and 20% fetal calf serum (Gibco). The cultures were maintained at 37°C with gas of 5% CO_2–95% air mixture. All media were supplemented with 5 U/ml heparin, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin. Medium was refreshed every 3 days. The endothelial cells were identified by the presence of factor VIII-related antigen (Histoset Kit, Immunolok, Carpinteria, CA) and typical "cobblestone" appearance. Endothelial cells of the third to fifth passages in actively growing condition were used for experiments. In experiments under high glucose situation, HUVECs were incubated in medium supplemented with glucose from basal level of 5.5–33 mM for different time intervals.

Evaluation of MMP-2, MMP-9, TIMP-1, and TIMP-2 by ELISA

For MMP activity assay, the Biotrack MMP Activity Assay System (Amersham Biosciences, Pharmacia) was used to detect total MMP-2 and MMP-9 activity, according to the manufacturer's instructions. The assay uses the pro form of a detection enzyme that can be activated by captured active MMP-2 or -9, into an active detection enzyme, through a single proteolytic event. MMP-activated detection enzyme can then be measured using a specific chromogenic peptide substrate. Total level of free MMP-2 or MMP-9 in cell culture supernatants (conditioned medium) was detected by including an incubation step using ρ-aminophenylmercuric acetate (APMA) to activate any bound MMP in its pro form. Active MMP-2 or -9 was detected without APMA treatment. Moreover, for the quantitative determination of TIMP-1 and TIMP-2 concentrations in conditioned medium, ELISA kits (Quantikine, R&D system, Minneapolis), based on the sandwich enzyme immunoassay technique, were employed. The monoclonal antibody specific for TIMP-1 or TIMP-2 has been pre-coated onto the microplates. Standards and samples were pipetted into the wells and any present TIMP-1 or TIMP-2 was bound by the immobilized antibody. Afterwards, washing any unbound substance, and an enzyme-linked polyclonal antibody specific for TIMP-1 or -2 was added to the wells. A prepared substrate solution was added to the wells and color developed in proportion to the amount of TIMP-1 or -2. Using a microplate reader set to 450 nm, we determined the optical density of each well with resultant color.

Gelatinolytic Zymography

Conditioned media from the equivalent cultured cells were analyzed by electrophoresis in the presence of 10% SDS–PAGE containing gelatin (1 mg/ml) which was prepared as described [Liotta and Stetler-Stevenson, 1990] with a minor modification. The gel was incubated in substrate buffer (5 mM $CaCl_2$, 100 mM NaCl, and 50 mM Tris, pH 6.8) containing 2.5% Trion X-100 at room temperature for 1 h, followed by further incubation in substrate buffer at 37°C for 18 h. The gelatinolytic activities were visualized by staining Coommasie Blue R-250 solution. Areas of protease activity will appear as clear bands against a dark blue background where the protease has digested the substrate.

Immunoblotting

Cellular lysates were prepared as previously described [Ho et al., 2000]. Specimens containing 60 μg of cellular lysate were subjected to electrophoresis on 10% SDS-polyacrylamide gels. The gels were then transferred onto nitrocellulose membranes and incubated in TBST buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 0.02% Tween 20) containing 5% nonfat milk. After blocking, the blots were incubated with TIMP-1, TIMP-2, or MT1-MMP antibodies (Santa Cruz Biotechnology, CA) in TBST for 1.5 h, followed by three washes (10 min each) with TBST buffer. The blots were then incubated with horseradish peroxidase-conjugated second antibodies for 30 min, and washed for three times (10 min each) with TBST buffer. Immunoreactivity was detected by enhanced chemiluminescence reagents according to manufacturer's instruction.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The total RNA was extracted with buffer containing 4 M guanidium isothiocyate, 25 mM sodium citrate, 0.5% lauroylsarcosine, and 0.7% β- mercaptoethanol. Reverse transcription was carried out in 50 μl reaction with 4 μg RNA and 2 μg random deoxynucleotide hexamers according to the manufacturer's instructions. The reaction was terminated by incubation of the samples at 94°C for 3 min. Specific cDNAs for MMP-2, MMP-9, TIMP-1, TIMP-2, MT1-MMP, and GAPDH were amplified with the ROBOCYCLER (Stratagene, La Jolla, CA). The optimal PCR results for the genes were obtained at an annealing temperature of 60°C with 35 cycles. The PCR products were resolved by agarose gel electrophoresis followed by staining with ethidium bromide. The oligonucleotide primers were all designed from the published sequence of the human genes: MMP-1 (sense), 5′-TTCATTTCTGTTTCTGGCC-3′; MMP-1 (antisense), 5′-ATTTTTCCTGCAGTTGAACC-3′ (462 bp) [Shiozawa et al., 2000]; MMP-2 (sense), 5′-ACCTGGATGCCGTCGTGGAC-3′; MMP-2 (antisense), 5′-TGTGGCAGCACCAGGGCAGC-3′ (447 bp) [Onisto et al., 1995]; MMP-9 (sense), 5-GGTCCCCCCACTGCTGGCCCTTCTACGGCC-3′; MMP-9 (antisense), 5-CACCTCCACTCCTCCCTTTCC-3′ (761 bp) [Wilhelm et al., 1989]; MT1-MMP (sense), 5′-GCCCATTGGCCAGTTCTGGCGGG-3; MT1-MMP (antisense), 5-CCTCGTCCACCTCAATGATGATC-3 (530 bp) [Lafleur et al., 2001]; TIMP1 (sense), 5-TGCACCTGTGTCCCACCCCACCCCACAGAC-3′; TIMP-1 (antisense), 5-GGCTACTGGGACCGCAGGGACTGCCAGGT-3′ (552 bp) [Carmichael et al., 1986]; TIMP-2 (sense), 5′-TGCAGCTGCTCCCCGGTGCAC-3′; TIMP-2 (antisense), 5′-TTATGGGTCCTCGATGTCGAG-3′ (590 bp); GAPDH (sense), 5′-CGGAGTCAACGGATTTGGTCGTAT-3′ [Onisto et al., 1995]; GAPDH (antisense), 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′ (307 bp) [Lafleur et al., 2001]. After amplification, 10 μl of each PCR reaction mix was subjected to electrophoresis through a 2% (w/v) agarose gel with ethidium bromide (0.5 μg/ml).

Detection of Cell Death

Apoptosis of the treated HUVECs was detected by the method of cell death detection ELISA (Boehringer Mannheim, Indianapolis, IN), as previously described [Ho et al., 2000]. This method is based on a quantitative sandwich-enzyme-immunoassay-principle. It can detect histone-associated DNA fragments in one immunoassay, demonstrating the internucleosomal degradation of genomic DNA occurring during apoptosis.

Statistical Analysis

Data were expressed as mean ± SEM from at least three independent experiments. Comparison of the multiple groups was performed by one way ANOVA followed by the student's

t-test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Identification of MMP-2 and MMP-9 Activity After High Glucose Treatment

To evaluate the activity of MMP-2 and -9 after high glucose (33 mM) treatment, HUVECs were incubated in high glucose-containing medium for different time periods (0–48 h). In Figure 1A-a, we found that the active MMP-2 level secreted in the conditioned media was gradually increased since 24 h after high glucose treatment. In contrast, total level of MMP-2 was not changed. In Figure 1B, the levels of total and active MMP-9 in the conditioned media were not significantly changed within 48 h treatment with high glucose. Moreover, using gelatinolytic zymography assay, we confirmed that the active form of MMP-2 (62 kDa) was increased in the conditioned media after high glucose treatment for 48 h, whereas the total MMP-2 level, which contains major pro-MMP-2, was not changed (Fig. 1A-b). In addition, using zymography assay, we did not detect the changes of MMP-9 level, either pro-form or active form, in high glucose-treated conditioned media (data not shown).

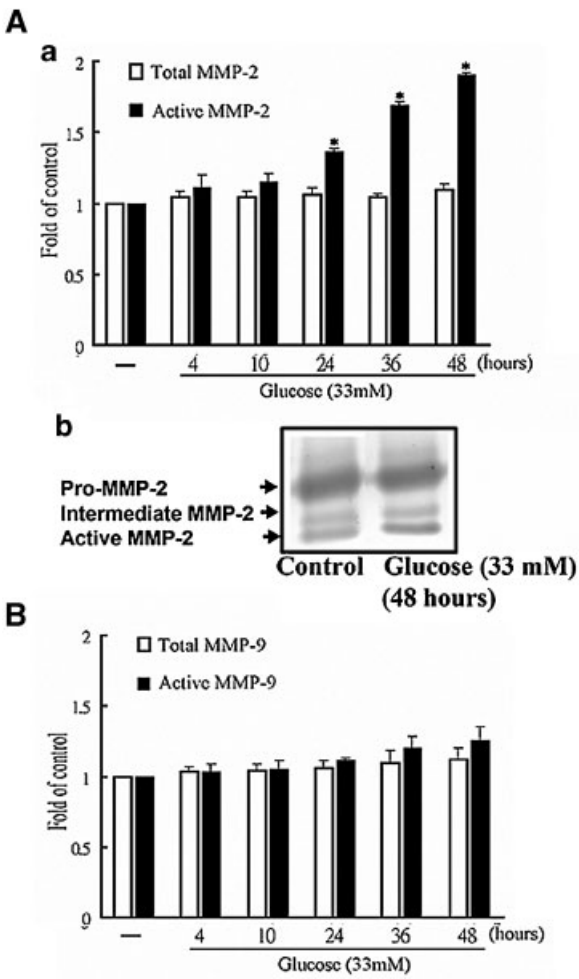

Fig. 1. The effects of high glucose on the levels of total and active MMP-2 and MMP-9 in HUVECs. HUVECs were treated with high glucose (33 mM) within 48 h. The total and active form of MMP-2 (**A-a**) and MMP-9 (**B**) were measured by ELISA kit as described in ''Materials and Methods.'' In **A-b**, the gelatinolytic activities from the conditioned media were examined by zymography. Bands of 72, 64, and 62 kDa indicate pro-MMP-2, intermediate MMP-2 and active MMP-2, respectively. All data are presented as mean ± SEM from five experiments performed in duplicate. $^*P < 0.05$ as compared with control group.

The Different Expression of TIMP-1 and TIMP-2 After High Glucose Treatment

In order to prove whether the endogenous tissue inhibitors of MMPs (TIMPs) were involved after high glucose treatment, the levels of TIMP-1 and TIMP-2 in the conditioned media were detected. We found that TIMP-1 level was not altered in conditioned media after high glucose treatment for 48 h, while TIMP-2 expression was time-dependently decreased (Fig. 2). At 10 h incubation with high glucose, TIMP-2 level decreased by about 50%, and continued to decrease to 10% after 48 h treatment.

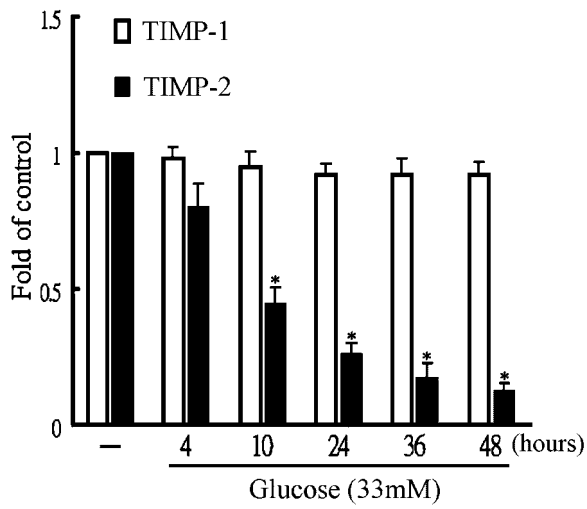

Fig. 2. The effects of high glucose on the levels of TIMP-1 and TIMP-2 in HUVECs. HUVECs were treated with high glucose (33 mM) for 4–48 h. The levels of TIMP-1 and TIMP-2 were measured by ELISA kit as described in ''Materials and Methods.'' All data are presented as mean ± SEM from five experiments performed in duplicate. $^*P < 0.05$ as compared with control group.

Influence of High Glucose on MMP-2, MMP-9, TIMP-1, TIMP-2, MT1-MMP mRNA, and Protein Expression

To understand the expression levels of MMP-2, MMP-9, TIMP-1, TIMP-2, MT1-TIMP protein, and mRNA after high glucose treatment, the methods of RT-PCR and Western Blot were used in different time periods (0–48 h). In Figure 3A-a, we analyzed the expression of MMP-2, MMP-9, MT1-MMP, and TIMPs mRNA after high glucose treatment with RT-PCR method. We found that the level of TIMP-2 mRNA after high glucose treatment was gradually decreased from 4 to 48 h. The levels of TIMP-1, MT1-MMP, MMP-2, and MMP-9 mRNA within 36 h did not change compared with the control group. However, the mRNA levels of TIMP-1, MT1-MMP, MMP-2, and MMP-9 were slightly decreased after high glucose treatment for 48 h. This effect might be related to the cell death at this time point. In Figure 3B, using Western Blotting analysis, we found that the expression of TIMP-2 protein after high glucose treatment was significantly decreased from 10 to 48 h. The expressions of TIMP-1 and MT1-TIMP proteins, however, were not changed. These data suggest that exposure of HUVECs to high glucose led to the specific inhibition of TIMP-2 mRNA expression accompanied by a reduction in TIMP-2 protein secretion.

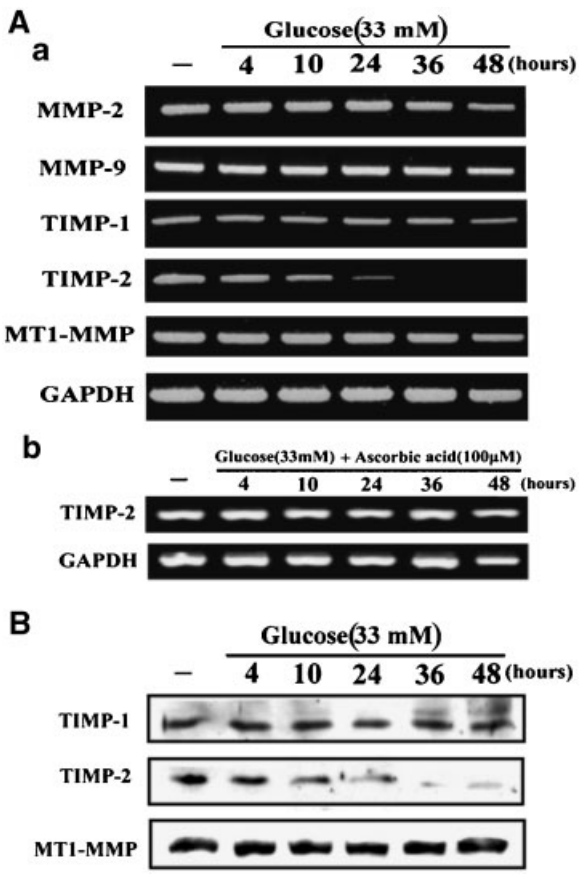

Fig. 3. The effects of high glucose on the mRNA and protein expressions of MMP-2, MMP-9, TIMP-1, TIMP-2, and MT1-MMP in HUVECs. HUVECs were treated with high glucose (33 mM) in the presence or the absence of ascorbic acid (100 μM) for 4–48 h. **A**: The mRNA expressions of MMP-2, MMP-9, TIMP-1, TIMP-2, and MT1-MMP were determined by RT-PCR method as described in ''Materials and Methods.'' **B**: The protein expressions of TIMP-1, TIMP-2, and MT1-MMP were determined by Western blot analysis using the specific antibodies for TIMP-1, TIMP-2, and MT1-MMP as described in ''Materials and Methods.'' All results are representative of at least four independent experiments.

Roles of Oxidative Stress in TIMP-2 and MMP-2 Activity

In our previous study, we demonstrated that high glucose could induce ROS production, which could be suppressed by ascorbic acid, an anti-oxidant agent. To understand the roles of ROS in high glucose-induced effects on MMPs and TIMPs, we treated cells with ascorbic acid (100 μM) together with high glucose. In Figure 4, we showed that the active form of MMP-2 after high glucose exposure for 48 h could be suppressed by ascorbic acid. The

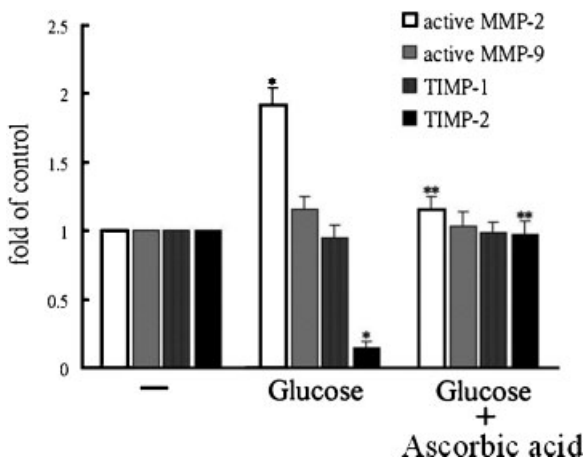

Fig. 4. Effects of ascorbic acid on the activity of MMP-2, MMP-9, TIMP-1, and TIMP-2 in HUVECs under high glucose treatment. HUVECs were treated with high glucose (33 mM) for 48 h in the presence or the absence of ascorbic acid (100 μM). Enzyme activities were determined by ELISA kits. All data are presented as mean ± SEM from five experiments performed in duplicate. $*P < 0.05$ as compared with control group. $**P < 0.05$ as compared with high glucose-treated group.

decrease of TIMP-2 expression was reversed by ascorbic acid as well. Neither the expression of MMP-9 or TIMP-1 was influenced after high glucose plus ascorbic acid treatment for 48 h. In accordance with this result, ascorbic acid can prevent high glucose-induced TIMP-2 mRNA reduction within 48 h incubation (Fig. 3A-b). These results suggest that the opposite effects on MMP-2 and TIMP-2 induced by high glucose are dependent on ROS production and can be reversed by ascorbic acid.

High Glucose-Induced Cell Apoptosis Is Mediated by Oxidative Stress and MMP Activation

Next, we attempted to evaluate the role of ROS and MMP/TIMP in cell apoptosis after high glucose exposure. HUVECs exposed to high glucose were treated with TIMP-1, TIMP-2, GM 6001 (a chemical synthetic and specific MMPs inhibitor), or ascorbic acid. In Figure 5A, we found that cell apoptosis induced by high glucose treatment for 48 h can be inhibited by ascorbic acid (100 μM). In Figure 5B, after high glucose treatment for 48 h, cell apoptosis can also be inhibited by GM 6001 (5 μM) and TIMP-2 (40 ng/ml), but not by TIMP-1(40 ng/ml). Treatment with GM 6001, TIMP-2 or TIMP-1 alone did not show significant toxicity in HUVECs. These results concluded that MMP activity is indeed involved in high glucose-mediated cell apoptosis.

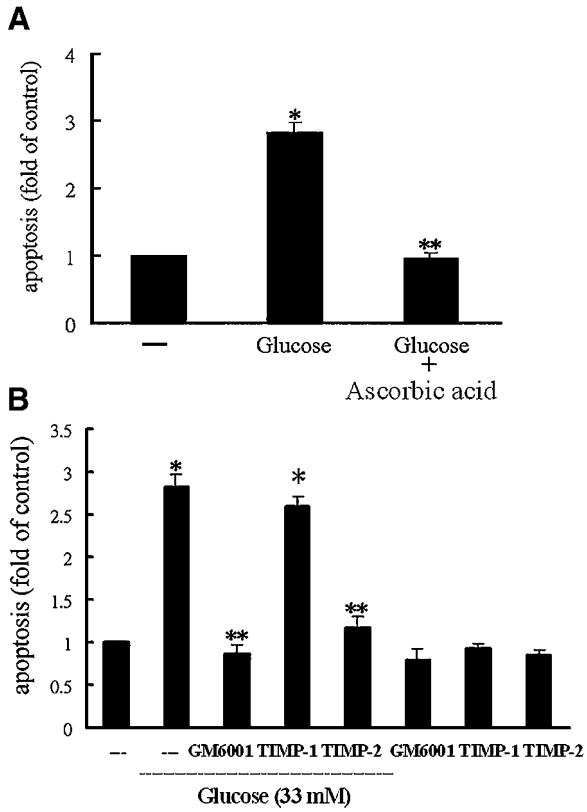

Fig. 5. Involvement of oxidative stress and MMP activation in high glucose-mediated HUVECs apoptosis. HUVECs were treated with high glucose (33 mM) for 48 h in the presence or the absence of ascorbic acid or other agents. **A**: Effect of ascorbic acid (100 μM) on the high glucose-induced cell apoptosis. **B**: Effects of GM6001 (5 μM), TIMP-1 (40 ng/ml), and TIMP-2 (40 ng/ml) on the high glucose-induced cell apoptosis. Cell apoptosis was determined by the method of cell death detection ELISA kit as described in "Materials and Methods." All data are presented as mean ± SEM from five experiments performed in duplicate. $*P < 0.05$ as compared with control group. $**P < 0.05$ as compared with high glucose-treated group.

DISCUSSION

In previous study, evidence showed that the activity of MMP can be induced by ROS [Rajagopalan et al., 1996]. Until now it still did not understand the role of MMPs in high glucose-induced cell apoptosis. In this study, we demonstrated that high glucose-induced HUVEC apoptosis is mediated by its opposite effects on MMP-2 and TIMP-2, leading to the increased MMP-2 activity.

MMPs are secreted by many types of cells as proenzymes. On activation by proteolytic cleavage, activated enzymes are capable of degrading many ECM components. The proteolytic activities of MMPs are tightly controlled during activation from their proenzymes to active forms by the combination of endogenous activators (e.g., MT-MMPs) and inhibitors. Proteinase and associated ECM degradation lead to apoptosis in several system involving development and cell death [Chen and Strickland, 1997]. Increased activity of MMPs has been implicated in numerous processes, including atherosclerosis and cardiovascular disease [Galis et al., 1994; Kai et al., 1998]. All vascular cells including endothelial cells and macrophages can secret MMPs. MMP-2 is predominantly secreted by endothelial cells [Nguyen et al., 2000], while the major MMP secreted from macrophages is the MMP-9 [Winberg et al., 2000]. The precursor of MMP-2 is tightly bound to TIMP-2, whereas MMP-9 proenzyme is associated with TIMP-1 [Goldberg et al., 1989]. The role of TIMPs in regulating matrix degradation may be exerted by protease elimination and blockage of MMPs activation.

Evidence demonstrated that glucose-induced expression of MMPs in several different cell types is mediated by a ROS-sensitive pathway [Rajagopalan et al., 1996]. The increase of MMP-9 activity after high glucose exposure was observed in cultured bovine endothelial cells [Uemura et al., 2001]. It has also been reported that high glucose exposure can induce the activity of MMP-2 in HUVECs [Death et al., 2003]. However, in current study, we found that MMP-2 activity, but not MMP-9, was increased in HUVECs culture after high glucose treatment. Moreover, the levels of MMP-2 and MMP-9 mRNA in HUVECs after high glucose exposure were not affected. These results were contrary to the findings by McLennan and colleagues that the increase of gene and protein expression of MMP-2 and decrease of MT1-MMP expression by high glucose were shown in human mesangial cells, demonstrating a good correlation of MMP-2 and MT-MMP expression [McLennan et al., 2000]. As shown in Figures 3 and 4 of present work, we did not observe such reciprocal changes between MMP-2 and MT1-MMP. We only found that the levels of TIMP-2 protein and mRNA in HUVECs after high glucose exposure were downregulated. Our current findings confirmed the report by Death and colleagues who showed that high glucose increased MMP-2 activity, while did not change TIMP-1 expression in HUVECs [Death et al., 2003]. Taken together, our current data suggest the activated MMP-2 is resulting from direct enzymatic activity rather than gene and protein upregulation. In contrast, the decreased TIMP-2 activity is resulting from the transcriptional downregulation of TIMP-2 mRNA, followed by the translational protein expression.

Much evidence was observed that MMPs play a key role in cell proliferation and angiogenesis and TIMPs can inhibit cell proliferation [Murphy et al., 1993]. Schnaper and colleagues demonstrated that the balance of MMPs and TIMPs is a primary determinant in the early stages of endothelial tube formation [Schnaper et al., 1993]. Instead addition of excess TIMP-1 or TIMP-2 can block endothelial tube formation in vitro. Despite previous studies on MMPs and TIMPs, it still has not elucidated regarding the causal relationship between apoptosis and balanced activity of MMP/TIMP in high glucose-stimulated cells. In the present study, we showed that a broad spectrum inhibitor of MMPs, GM 6001, and a specific inhibitor TIMP-2, but not TIMP-1, can inhibit HUVECs apoptosis induced by high glucose exposure. These results, therefore, suggest that the high glucose-induced endothelial cell apoptosis is mediated not only by the increase of active form MMP-2 but also by the decrease of its endogenous inhibitor TIMP-2. One of the interesting finding, as shown in Figure 4, is that the high glucose-induced opposite effects on MMP-2 and TIMP-2 in HUVECs can be reversed by ascorbic acid. This finding suggests that ROS production following high glucose plays an important role to mediate TIMP-2 gene expression, accompanied by the regulation of MMP-2 activity. As a result, this MMP-2-dependent cell apoptosis is dependent on ROS production.

Our previous study demonstrated that NO plays a protective role from apoptosis of HUVECs during the early stage of high glucose exposure, but in the late stage, high glucose exposure leads to the imbalance of NO and ROS, resulting to the observed apoptosis [Ho et al., 1999]; further findings showed that ROS induced by high glucose may be involved in c-Jun N-terminal kinase (JNK) activation, which in turn triggers the caspase-3 that facilitates the apoptosis in HUVECs [Ho et al., 2000]. Moreover, our recent results demonstrated that high glucose-induced HUVECs apoptosis is through NF-κB-dependent JNK activation and ROS-dependent Akt dephosphorylation [Ho et al., 2006]. Activated Akt/PKB, which is a core component of the phosphoinositide 3-kinase (PI3K) signaling pathway, is a powerful promoter of cell survival, as it antagonizes apoptosis by phosphorylating and inactivating various components of the apoptotic machinery. PI3K/Akt signals have also been reported to directly involve the regulation of expression of MMP-2 and -9 that related to the cancer cell invasion [Kim et al., 2001; Sun et al., 2006]. Therefore, the role of PI3K/Akt signals in the opposite effects on MMP-2 and TIMP-2 in human endothelial cells and its related apoptosis after exposure to high glucose should be subjected to further investigation.

In conclusion, high glucose-induced oxidative stress seems to play an important role in the regulation of MMP-2 activity. Moreover, endothelial cell apoptosis induced by high glucose is mediated not only by an increase of MMP-2 activity, but also accompanied by a

decrease of TIMP-2 expression. From these findings, we suggest that normalization of the balance between MMP-2 and TIMP-2, and inhibition of ROS production may yield therapeutic strategies for hyperglycemia-related atherosclerosis. Moreover, in our study, we found that GM6001 could markedly inhibit high glucose-induced HUVEC apoptosis. This result further implies that the synthetic MMPs inhibitor(s) may have potential in preventing hyperglycemia-related atherosclerosis. The in vivo studies stay to be confirmed in the future.

ACKNOWLEDGMENTS

This work was supported by the National Science Council of Taiwan (NSC 95-2314-B-087-001).

REFERENCES

Borden P, Heller RA. 1997. Transcriptional control of matrix metalloproteinases and tissue inhibitors of metalloproteinases. Crit Rev Eukaryot Gene Exp 7: 159–178.

Carmichael DF, Sommer A, Thompson RC, Anderson DC, Smith CG, Welgus HG, Stricklin GP. 1986. Primary structure and cDNA cloning of human fibroblast collagenase inhibitor. Proc Natl Acad Sci USA 83(8):2407–2411.

Chen ZL, Strickland S. 1997. Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. Cell 91(7):917–925.

Creemers EE, Cleutjens JP, Smits JF, Daemen MJAP. 2001. Matrix metalloproteinase inhibition after myocardial infarction: A new approach to prevent heart failure? Circ Res 89:201–210.

Death AK, Fisher EJ, McGrath KCY, Yue DK. 2003. High glucose alters matrix metalloproteinase expression in two key vascular cells: Potential impact on atherosclerosis in diabetes. Atherosclerosis 168:263–269.

Fisher E, McLennan SV, Tada H, Heffernan S, Yue DK, Turtle JR. 1991. Interaction of ascorbic acid and glucose on the production of collagen and proteoglycan by fibroblasts. Diabetes 40:371–376.

Galis ZS, Sukhova GK, Lark MW, Libby P. 1994. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 94:2493–2503.

Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Libby P. 1995. Enhanced expression of vascular matrix metalloproteinases induced in vitro by cytokines and in regions of human atherosclerotic lesions. Ann New York Acad Sci 748:501–507.

Goldberg GI, Marmer BL, Grant GA, Eisen AZ, Wilhelm S, He CS. 1989. Human 72 kilodalton type IV collagenase forms a complex with a tissue inhibitor of metalloproteases designated TIMP-2. Proc Natl Acad Sci USA 86: 8207–8211.

Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. 1997. Tissue inhibitors of metalloprteinases: Structure, regulation and biological function. Eur J Cell Biol 74:111–122.

Ho FM, Liu SH, Liau CS, Huang PJ, Shiah SG, Lin-Shiau SY. 1999. Nitric oxide prevents apoptosis of human endothelial cells from high glucose exposure during early stage. J Cell Biochem 75:258–263.

Ho FM, Liu SH, Liau CS, Huang PJ, Lin-Shiau SY. 2000. High glucose-induced apoptosis in human endothelial cells is mediated by sequential activations of c-Jun NH_2-terminal kinase and caspase-3. Circulation 101:2618–2624.

Ho FM, Lin WW, Chen BC, Chao CM, Yang CR, Lin LY, Lai CC, Liu SH, Liau CS. 2006. High glucose-induced apoptosis in human vascular endothelial cells is mediated through NF-κB and c-Jun NH_2-terminal kinase pathway and prevented by PI3K/Akt/eNOS pathway. Cell Signal 18:391–399.

Kai H, Ikeda H, Yasukawa H, Kai M, Seki Y, Kuwahara F, Ueno T, Sugi K, Imaizumi T. 1998. Peripheral blood levels of matrix metalloproteinases-2 and -9 are elevated in patients with acute coronary syndromes. J Am Coll Cardiol 32:368–372.

Kannel WB, Mcgee DL. 1979. Diabetes and glucose tolerance as risk factors for ca rdiovascular disease: The Framingham study. Diabetes Care 2:120–126.

Kim D, Kim S, Koh H, Yoon SO, Chung AS, Cho KS, Chung J. 2001. Akt/PKB promotes cancer cell invasion via increased motility and metalloproteinase production. FASEB J 15:1953–1962.

Komorowski J, Pasicka Z, Jankiewicz-Wika J, Stepien H. 2002. Matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases and angiogenic cytokines in peripheral blood of patients with thyroid cancer. Thyroid 12:655–662.

Lafleur MA, Hollenberg MD, Atkinson SJ, Knauper V, Murphy G, Edwards DR. 2001. Activation of pro-matrix metalloproteinase-2 (pro-MMP-2) by thrombin is membrane-type-MMP-dependent in human umbilical vein cells and generates a distinct 63 kDa active species. Biochem J 357(Pt 1):107–115.

Liotta LA, Stetler-Stevenson WG. 1990. Metalloproteinases and cancer invasion. Semin Cancer Biol 2:96–106.

McLennan SV, Martell SYK, Yue DK. 2000. High glucose concentration inhibits the expression of membrane type metalloproteinase by mesangial cells: Possible role in mesangium accumulation. Diabetologia 43:642–648.

Murphy AN, Unsworth EJ, Stetler-Stevenson WG. 1993. Tissue inhibitor of metalloproteinase-2 inhibits bFGF-induced human microvascular endothelial cell proliferation. J Cell Physiol 157:351–358.

Nagase H. 1997. Activation mechanisms of matrix metalloproteinases. Biol Chem 378:151–160.

Nagase H, Woessner JF. 1999. Matrix metalloproteinases. J Biol Chem 274:21491–21494.

Nguyen M, Arkell J, Jackson C. 2000. Activated protein kinase C directly activates human endothelial gelatinase A. J Biol Chem 275:9095–9098.

Onisto M, Riccio MP, Scannapieco P, Caenazzo C, Griggio L, Spina M. Stetler-Stevenson WG, Garbisa S. 1995. Gelatinase A/TIMP-2 imbalance in lymph-node-positive breast carcinomas, as measured by RT-PCR. Int J Cancer 63:621–626.

Puliese G, Pricci F, Pugliese F, Mene P, Lenti L, Andreani D, Galli G, Casini A, Bianchi S, Rotella CM. 1994. Mechansims of glucose-enhanced extracellular matrix

accumulation in rat glomerular mesangial cells. Diabetes 43:478–490.

Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. 1996. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 98:2572–2579.

Rudermam NB, Haudenchild C. 1984. Diabetes as an atherogenic factor. Prog Cardiovasc Dis 26:373–412.

Schnaper HW, Grant DS, Stetler-Stetler-Stevenson WG, Fridman R, D'Orazi GD, Murphy A, Bird RE, Hoythya M, Fuerst TR, French DL. 1993. Type IV collagenases and TIMPs modulate endothelial cell morphogenesis in vitro. J Cell Physiol 156:235–246.

Shiozawa J, Ito M, Nakayama T, Nakashima M, Kohno S, Sekine I. 2000. Expression of matrix metalloproteinase-1 in human colorectal carcinoma. Mod Pathol 13:925–933.

Sun LC, Luo J, Mackey LV, Fuselier JA, Coy DH. 2006. A conjugate of camptothecin and a somatostatin analog against prostate cancer cell invasion via a possible signaling pathway involving PI3K/Akt, alphaVbeta3/alphaVbeta5 and MMP-2/-9. Cancer Lett (in press).

The Diabetes Control and Complications Trial Research Group. 1993 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New Engl J Med 329:977–986.

Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee K-H, Harrison DG, Tsao PS. 2001. Diabetes mellitus enhances vascular matrix metalloproteinase activity: Role of oxidative stress. Circ Res 88:1291–1298.

Wilhelm SM, Collier IE, Marmer BL, Eisen AZ, Grant GA, Goldberg GI. 1989. SV40-transformed human lung fibroblasts secreted a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophages. J Biol Chem 264:17213–17221.

Winberg J, Kolset SO, Bergh E, Uhlin-Hansen L. 2000. Macrophages secrete matrix netalloproteinase 9 covalently linked to the core protein of chondroitin sulphate proteoglycans. J Mol Biol 304:669–680.