J Periodont Res 2004; 39; 415–423
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JOURNAL OF PERIODONTAL RESEARCH
doi: 10.1111/j.1600-0765.2004.00756.x

# Pre-exposure to high glucose augments lipopolysaccharide-stimulated matrix metalloproteinase-1 expression by human U937 histiocytes

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*Maldonado A, He L, Game BA, Nareika A, Sanders JJ, London SD, Lopes-Virella MF, Huang Y. Pre-exposure to high glucose augments lipopolysaccharide-stimulated matrix metalloproteinase-1 expression by human U937 histiocytes. J Periodont Res 2004; 39; 415–423. © Blackwell Munksgaard 2004*

*Background and objectives:* It has been well established that patients with diabetes have increased prevalence and severity of periodontal diseases. However, the underlying mechanisms are not well understood. Given that bacterial infection is the primary cause of periodontal disease, we postulated that hyperglycemia may interplay with bacterial virulence factors such as lipopolysaccharide to up-regulate matrix metalloproteinase (MMP), leading to increased periodontal tissue destruction.

*Methods and results:* We showed that prolonged pre-exposure of U937 histiocytes to high glucose markedly increased lipopolysaccharide-stimulated MMP-1 secretion and mRNA expression. Our results also showed that the effect of high glucose on lipopolysaccharide-induced MMP-1 expression is cell type-specific because no similar response was observed in human gingival fibroblasts. In addition to MMP-1, high glucose also augments lipopolysaccharide-stimulated MMP-7, -8, and -9 mRNA expression. In the investigation of the signaling pathways involved in the enhancement of lipopolysaccharide-induced MMP-1 expression by high glucose, we found that both high glucose and lipopolysaccharide regulate MMP-1 expression through the nuclear factor κB (NFκB) and mitogen-activated protein kinase (MAPK) cascades.

*Conclusions:* The present study has shown that pre-exposure to high glucose and subsequent lipopolysaccharide treatment synergistically stimulates MMP-1 expression by mononuclear phagocytes through the NFκB and MAPK signaling pathways. This study has thus delineated a pathogenic mechanism that may be involved in the exacerbated periodontal disease in diabetic patients.

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Key words: diabetes mellitus; gene expression; glucose; lipopolysaccharide; macrophages; matrix metalloproteinases; periodontal diseases; signal transduction

Accepted for publication May 24, 2004

Epidemiological studies have well documented that patients with either type 1 or type 2 diabetes have increased prevalence and severity of periodontal diseases (1). Indeed, periodontal disease has been recognized as another complication of diabetes (2). To understand the pathogenesis of diabetes-associated periodontal disease, several mechanisms such as impaired host defense to bacterial pathogens, increased susceptibility to infection, and decreased collagen production have been proposed previously (1). In recent years, the role of protein glycosylation in periodontal disease has been studied considerably and it suggests that advanced glycosylation endproducts contribute to accelerated periodontal disease in diabetes (3, 4). Thus, it is generally accepted that multiple mechanisms are involved in the progression of diabetes-associated periodontal disease (4).

The primary cause of periodontal disease is bacterial infection (5), which is different from that of other complications including atherosclerosis, nephropathy, retinopathy, neuropathy, and peripheral vascular disease. Periodontopathic bacteria exhibit a number of virulence factors, such as lipopolysaccharide, lipoteichoic acids, toxins, proteinases and short-chain fatty acid, which interact with mononuclear phagocytes and elicit inflammatory responses (6). Chronic infection and inflammation often leads to advanced periodontal diseases that are clinically characterized by the destruction of the connective tissue attachment to the root of the tooth, loss of alveolar bone, and pocket formation (1).

Given the crucial role of bacterial virulence factors in periodontal disease, it is likely that the diabetes-associated pathogenic factors such as hyperglycemia interplay with bacterial virulence factors to promote the progression of periodontal disease in diabetes. In the present study, we have studied the effect of pre-exposure of U937 histiocytes (resident macrophages) to high concentration of glucose on lipopolysaccharide-stimulated matrix metalloproteinase (MMP)-1 expression. Results showed that high glucose markedly augments lipopolysaccharide-stimulated MMP-1 expression.

## Material and methods

### Cell culture

U937 histiocytes (7) (American Type Culture Collection, Manassas, VA, USA) were cultured in a 5% $CO_2$ atmosphere in F-10 Nutrient Mixture medium (Gibco, Invitrogen Corp., Carlsbad, CA, USA) containing 10% fetal calf serum and 5 or 25 mM of D-glucose. The glucose concentrations of 5 and 25 mM were designated as normal and high glucose, respectively. The medium was changed every 2–3 days. Histiocyte is also called resident macrophage (8) and the histiocytic origin of U937 cells was shown by its capacity for lysozyme production and the strong esterase activity (7). Human gingival HGF-1 fibroblasts (American Type Culture Collection) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 5 or 25 mM of D-glucose. The medium was changed every 2–3 days. U937 cells were treated with lipopolysaccharide (Sigma, St. Louis, MO, USA) that was highly purified from *Escherichia coli* by phenol extraction and gel filtration chromatography, and was cell culture tested.

### Enzyme-linked immunosorbent assay

MMP-1 and tissue inhibitor of metalloproteinase-1 (TIMP-1) (9) secreted into culture medium by U937 cells were quantified using sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the protocol provided by the manufacturer (R & D System, Minneapolis, MN, USA).

### Collagenase activity assay

Collagenase activity in conditioned medium was determined with the EnzChek assay kit according to the protocol provided by the manufacture (Molecular Probes, Eugene, OR, USA).

### Real-time polymerase chain reaction

Total RNA was isolated from cells using a RNeasy minikit (Qiagen, Santa Clarita, CA, USA). First-strand complementary DNA (cDNA) was synthesized with the iScript$^{TM}$ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) using a 20 µl reaction mixture containing 0.75 µg of total RNA, 4 µl of 5 × iScript Reaction Mixture, 1 µl iScript Reverse Transcriptase. The complete reaction was then cycled for 5 min at 25°C, 30 min at 42°C and 5 min at 85°C using a PTC-200 DNA Engine (MJ Research, Waltham, MA, USA). The reverse transcription reaction mixture was then subjected to polymerase chain reaction (PCR) amplification of MMP-1 cDNA using sense primer 5′-CTGGGAAGCCATCACTTACCTTGC-3′ and antisense primer 5′-GTTTCTAGAGTCGCTGGGAAGCTG-3′. The Beacon Designer Software (PREMIER Biosoft International, Palo Alto, CA, USA) was used for primer designing. Primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA, USA). PCR was carried out in duplicates using a 25 µl reaction mixture that contained 1.5 µl reverse transcription reaction mixture, 0.2 µM of both primers and 12.5 µl of iQ$^{TM}$ SYBR Green Supermix (Bio-Rad). The PCR reaction was performed using the iCycler$^{TM}$ Real-Time Detection System (Bio-Rad) with a two-step method, hot-start enzyme activated (95°C for 3 min), then amplified for 40 cycles consisting of denaturation (95°C for 10 s) and annealing/extension (56°C for 45 s). A melt-curve was then subsequently performed (55°C for 1 min and then temperature is increased by 0.5°C every 10 s). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified with the primers 5′-GAA TTTGGCTACAGCAACAGGGTG-3′ and 5′-TCTCTTCCTCTTGTGCTCTTGCTG-3′. Amplicon size and reaction specificity were confirmed by 2.5% agarose gel electrophoresis. Data were analyzed with the SmartCycler II software. The average $C_t$ (threshold cycle) of fluorescence units was used for analysis. Quantification was calculated using the $C_t$ of the target signal relative to the GAPDH signal in the same RNA sample.

### Gene array analysis

Total RNA was isolated from cells as described above. MMP mRNA array analysis was performed with a gene array kit (SuperArray Bioscience Corp., Frederick, MD, USA) by following the instructions provided by the manufacturer. Briefly, reverse transcription reaction was conducted first to convert mRNA to cDNA that was used as template for PCR. PCR was conducted in the presence of biotin-16-dUTP for 30 cycles with each cycle containing three temperatures: 85°C for 1 min, 50°C for 1 min, and 72°C for 1 min. The membranes arrayed with 96 cDNAs of extracellular matrix and adhesion molecule genes were then hybridized with amplified and biotin-labeled cDNA probes. After washing, the membranes were incubated with alkaline phosphatase-conjugated streptavidin and CDP-Star chemiluminescent substrate. The cDNA was visualized by exposure of the membranes to X-ray film for 1 min and the data were analyzed using a software (GEArray Analyzer) provided by the manufacturer.

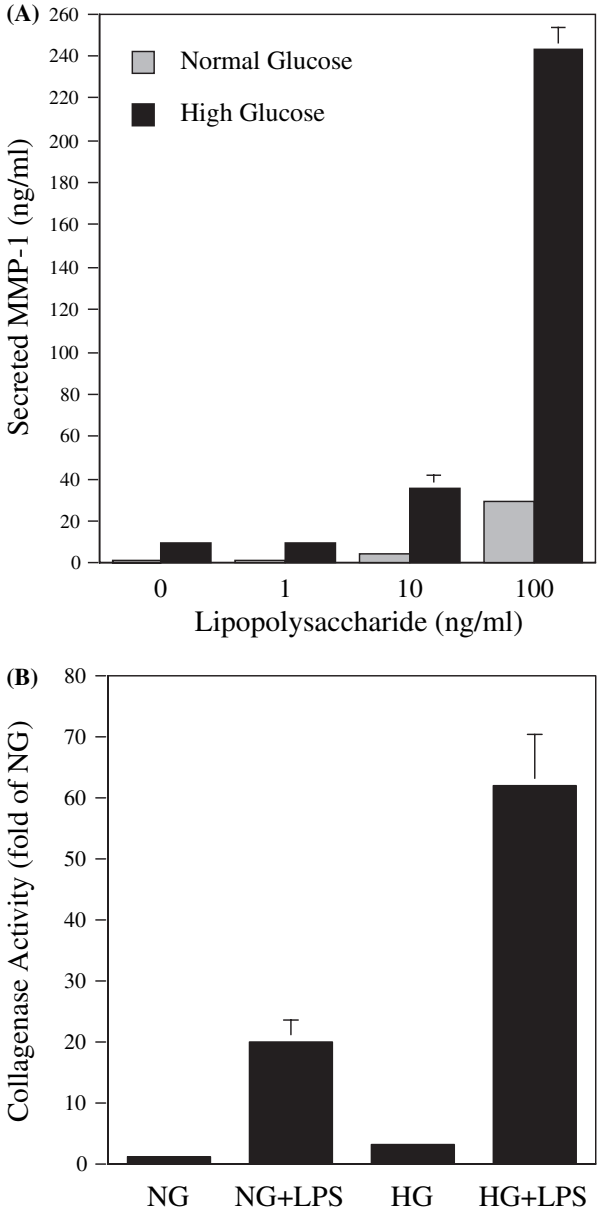


*Fig. 1.* The effect of pre-exposure to high glucose on lipopolysaccharide (LPS)-stimulated matrix metalloproteinase-1 (MMP-1) secretion. U937 cells were cultured in medium containing normal glucose (5 mM, gray bars) or high glucose (25 mM, black bars) for 4 weeks and then treated with different concentrations (0–100 ng/ml) of LPS for 24 h. After the treatment, secreted MMP-1 in the conditioned medium was quantified by ELISA (A) and collagenase activity was determined by collagenase activity assay (B). Data (mean ± SD) presented are the representative of three experiments with similar results.

### Blocking of signal transduction pathways

U937 cells were treated with lipopolysaccharide in the presence or absence of the inhibitors of the nuclear factor kB (NFκB) and mitogen-activated protein kinase MAPK [c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38] (10), including BAY 11-7085, a specific inhibitor of NFκB pathway (11), and SP600125 (12), PD98059 (13), and SB203580 (14, 15), specific inhibitors of JNK, ERK and p38 pathways, respectively. After the incubation, the amount of MMP-1 in the conditioned medium was quantified by ELISA.

### Statistical analysis

Data were presented as mean ± standard deviation. Comparison between treatments was performed using the one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered significant.

## Results

### Prolonged pre-exposure of U937 histiocytes to high glucose enhances lipopolysaccharide-stimulated matrix metalloproteinase-1 secretion

The circulating mononuclear phagocytes in diabetic patients with hyperglycemia are chronically exposed to high level of serum glucose. Thus, we attempted to determine the effect of prolonged pre-exposure of mononuclear phagocytes to high glucose on lipopolysaccharide-stimulated MMP-1

expression. For this purpose, we cultured human U937 histiocytes in medium containing normal (5 mM) or high glucose (25 mM) for 4 weeks before treatment with different concentrations (0, 1, 10 and 100 ng/ml) of lipopolysaccharide for 24 h. The secreted MMP-1 in conditioned medium was quantified by ELISA. Results (Fig. 1A) showed that without lipopolysaccharide treatment, high glucose increased MMP-1 secretion by eightfold when compared to normal glucose (9.6 vs. 1.2 ng/ml, $p < 0.01$). Results also showed that lipopolysaccharide stimulated MMP-1 secretion by both normal glucose- and high glucose-treated cells in a concentration-dependent manner. Interestingly, the amount of MMP-1 secreted by high glucose-cultured cells in response to lipopolysaccharide was seven- to eightfold of that secreted by normal glucose-cultured cells (35.4 vs. 5.0 ng/ml in response to 10 ng/ml of lipopolysaccharide and 243.2 vs. 29.6 ng/ml in response to 100 ng/ml of lipopolysaccharide, $p < 0.01$), suggesting that pre-exposure to high glucose markedly augments lipopolysaccharide-stimulated MMP-1 secretion. To determine if the augmentation of lipopolysaccharide-stimulated MMP-1 secretion by high glucose contributes to lipopolysaccharide-induced collagenase activity, collagenase activity in conditioned medium was determined. Our data showed that the treatment of cells with high glucose for 4 weeks led to a threefold increase in collagenase activity when compared to that with normal glucose (Fig. 1B).

### Prolonged pre-exposure to high glucose is required for augmentation of lipopolysaccharide-stimulated matrix metalloproteinase-1 secretion

To determine if pre-exposure of cells to high glucose is required for augmentation of lipopolysaccharide-stimulated MMP-1 secretion, U937 cells, which had been cultured in normal glucose-containing medium, were treated with high glucose plus 100 ng/ml of lipopolysaccharide at the same time for 24 h. Results showed that high glucose did not augment lipopolysaccharide-stimulated MMP-1 secretion as compared to normal glucose (Fig. 2), suggesting that pre-exposure to high glucose is necessary for augmentation of lipopolysaccharide-stimulated MMP-1 secretion. Time course study was also conducted to determine the time for the pre-exposure to high glucose that is optimal for the augmentation. Results (Table 1) showed that the augmentation of lipopolysaccharide-stimulated MMP-1 secretion by high glucose was time-dependent and that the augmentation reached seven- to eightfolds after cells were exposed to high glucose for 4 weeks.

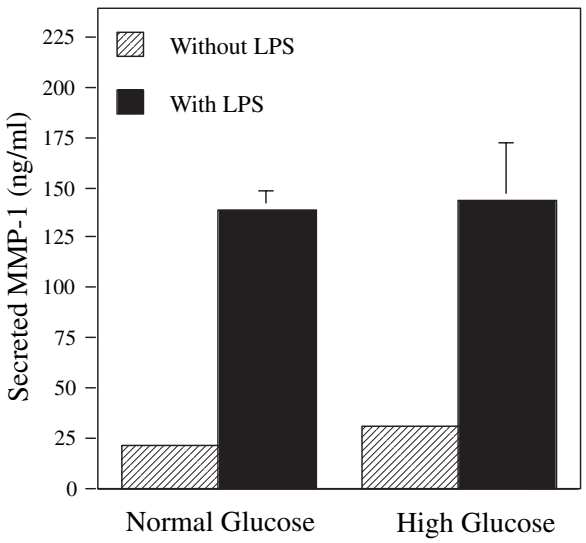


*Fig. 2.* The effect of high glucose on lipopolysaccharide (LPS)-stimulated matrix metalloproteinase-1 (MMP-1) secretion when cells were exposed to high glucose and LPS simultaneously. U937 cells cultured in normal glucose (5 mM)-containing medium for 4 weeks were treated with normal (5 mM, gray bars) or high glucose (25 mM, black bars) in the presence or absence of LPS (100 ng/ml) for 24 h. After the treatment, secreted MMP-1 in the conditioned medium was quantified by ELISA. Data (mean ± SD) presented are the representative of three experiments with similar results.

### High glucose moderately increases lipopolysaccharide-stimulated tissue inhibitor of metalloproteinase-1 secretion

The effect of high glucose and lipopolysaccharide on the secretion of TIMP-1, a natural inhibitor of MMP-1 (9), by U937 cells was also determined. Results (Fig. 3) showed that in the absence of lipopolysaccharide, high glucose alone did not increase TIMP-1. lipopolysaccharide had no effect on TIMP-1 secretion from normal glucose-cultured cells, but lipopolysaccharide at 10 and 100 ng/ml stimulated TIMP-1 secretion from high glucose-cultured cells by twofold as compared to that secreted from normal glucose-cultured cells. These data indicate that high glucose moderately increased lipopolysaccharide-stimulated TIMP-1 secretion as compared to normal glucose. Obviously, high glucose increases more MMP-1 secretion than TIMP-1 (seven- vs. twofold) from cells in response to lipopolysaccharide.

### High glucose has no effect on lipopolysaccharide-stimulated matrix metalloproteinase-1 secretion by gingival fibroblasts

The effect of pre-exposure to high glucose on lipopolysaccharide-stimulated MMP-1 secretion by gingival fibroblasts was also determined. Results (Fig. 4) showed that in contrast to U937 cells, high glucose alone inhibited MMP-1 secretion as compared to normal glucose. Although lipopolysaccharide stimulates MMP-1 secretion in both normal and high glucose-treated cells, no increase in MMP-1 secretion in cells pre-exposed to high glucose as

*Table 1.* The relationship between the time for high glucose exposure and the increase in the augmentation of lipopolysaccharide (LPS)-stimulated matrix metalloproteinase-1 (MMP-1) secretion

| Weeks for high glucose exposure | Fold increase in MMP-1 secretion by high glucose-treated cells as compared to that by normal glucose-treated cells |
|---|---|
| 1 | Not significant |
| 2 | 2 |
| 3 | 3–4 |
| 4 | 7–8 |
| 5 | 7–8 |

U937 cells were cultured in normal (5 mM) or high glucose (25 mM)-containing medium for different time as indicated before the treatment with 100 ng/ml of LPS for 24 h. MMP-1 secreted into medium was quantifies by ELISA. The ratio of the amount of secreted MMP-1 by cells pre-exposed to high glucose vs. that by cells pre-exposed to normal glucose was calculated.

compared to cells pre-exposed to normal glucose was observed. Thus, these data suggest that high glucose-enhanced MMP-1 secretion in response to lipopolysaccharide is cell type-specific.

### High glucose enhances lipopolysaccharide-stimulated matrix metalloproteinase-1 mRNA expression

To determine if high glucose-enhanced MMP-1 secretion is due to increased MMP-1 mRNA expression, cellular MMP-1 mRNA level was determined by quantitative real-time PCR (Fig. 5A). As an internal control, GAPDH mRNA was also determined by real-time PCR (Fig. 5B). Results showed that high glucose increased MMP-1 mRNA by fivefold and augmented lipopolysaccharide-stimulated MMP-1 mRNA expression by fourfold when compared to normal glucose (Fig. 5C), which is similar to the results from ELISA (Fig. 1). These data suggest that high glucose-enhanced MMP-1 secretion by cells in response to lipopolysaccharide is due to the increased MMP-1 mRNA expression.

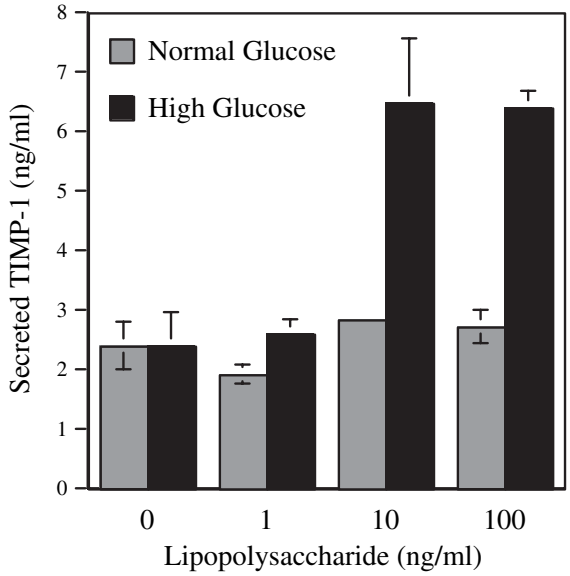


*Fig. 3.* The effect of pre-exposure to high glucose on lipopolysaccharide (LPS)-stimulated tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) secretion. U937 cells were cultured in medium containing normal glucose (5 mM, gray bars) or high glucose (25 mM, black bars) for 4 weeks and then treated with different concentrations (0–100 ng/ml) of LPS for 24 h. After the treatment, secreted TIMP-1 in the conditioned medium was quantified by ELISA. Data (mean ± SD) presented are the representative of three experiments with similar results.

### High glucose also enhances lipopolysaccharide-stimulated matrix metalloproteinase-7, -8, and -9 mRNA expression

To determine if high glucose also increases mRNA levels of other MMPs, we performed DNA array hybridization analysis. Results (Table 2) showed that in addition to MMP-1, high glucose also increased MMP-7, -8, and -9 mRNA expression, and augmented lipopolysaccharide-stimulated mRNA levels of MMP-7, -8, and -9 mRNA level by three-, two-, and threefold, respectively, when compared to normal glucose.

### High glucose and lipopolysaccharide regulate matrix metalloproteinase-1 expression through multiple signaling pathways

The above studies show a synergistic effect of high glucose and lipopolysaccharide on MMP-1 expression by U937 cells. To understand the underlying mechanisms, we investigated the signaling pathways potentially involved in the stimulation of MMP-1 by high glucose and lipopolysaccharide. As previous studies have shown that lipopolysaccharide regulates gene expression through NFκB and MAPK signaling pathways (10), we performed blocking studies using specific inhibitors for these pathways. Results showed that 10 μM of BAY 11-7085, a specific inhibitor of NFκB pathway (11), markedly inhibited the stimulation of MMP-1 secretion by either lipopolysaccharide or high glucose (Figs 6A and B). Results also showed that 10 μM of SP600125 (12), PD98059 (13), and SB203580 (14, 15), specific inhibitors of JNK, ERK and p38 pathways, respectively, significantly attenuated the stimulation by either lipopolysaccharide- or high glucose-stimulated MMP-1 secretion (Figs 6A and B). Furthermore, all these inhibitors were found to effectively inhibit the enhancement of lipopolysaccharide-stimulated MMP-1 secretion by high glucose in a similar manner (Fig. 6C).

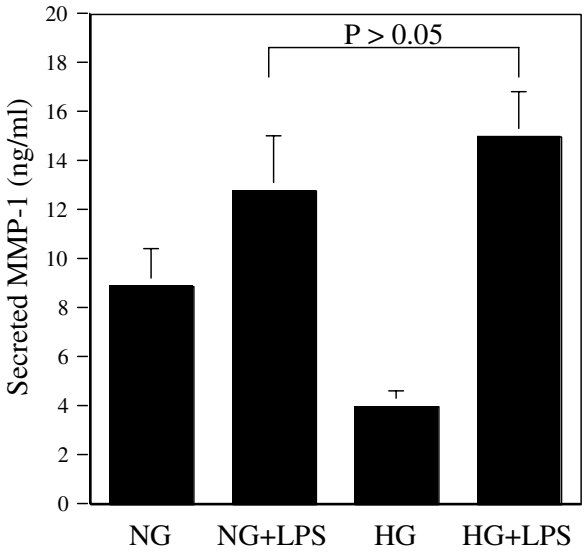


*Fig. 4.* The effect of pre-exposure to high glucose on lipopolysaccharide (LPS)-stimulated matrix metalloproteinase-1 (MMP-1) secretion by gingival fibroblasts. Human HGF-1 gingival fibroblasts were pre-exposed to normal glucose (5 mM) or high glucose (25 mM) for 4 weeks and then treated with 100 ng/ml of LPS for 24 h. After the treatment, secreted MMP-1 in the conditioned medium was quantified by ELISA. Data (mean ± SD) presented are the representative of three experiments with similar results.

Thus, these data suggest that multiple cascades including NFκB and MAPK (JNK, ERK and p38) pathways are involved in the synergistic effect of high glucose and lipopolysaccharide on the MMP-1 up-regulation.

## Discussion

Periodontal disease is characterized by connective tissue destruction (1). Previous studies have shown that collagenases, including MMP-8 (neutrophil-type collagenase) and MMP-1 (fibroblast-type collagenase), contribute to periodontal tissue destruction (16, 17). Although MMP-8 has been considered to be the major collagenase for the collagen degradation (17), several lines of evidence indicate that MMP-1 also plays an important role in periodontal disease (16, 18, 19). First, it has been shown that increased MMP-1 mRNA expression was correlated with decreased gingival collagen in patients with periodontal disease (18). Second, MMP-1 protein level in gingival crevicular fluid collected from patients with chronic periodontitis was found to be higher than that from periodontally healthy individuals (19). Third, a recent study demonstrated that polymorphism in the promoter region of MMP-1 gene, which leads to increased MMP-1 production, is associated with severe chronic periodontitis phenotype in non-smokers (20). Thus, MMP-1 released by mononuclear phagocytes and gingival fibroblasts is likely involved in periodontal tissue destruction.

It is known that lipopolysaccharide is a potent stimulator of MMP expression by mononuclear cells (21, 22). Since the Gram-negative bacteria is the major pathogen involved in periodontal disease, it is generally believed that lipopolysaccharide, a membrane component of the Gram-negative bacteria, is involved in the destructive periodontal disease by increasing MMP production from mononuclear phagocytes (6). However, it is not clear how lipopolysaccharide regulates MMP expression under normal and high glucose conditions. Because it has been shown that hyperglycemia has profound effects on gene expression (23), we postulated that high glucose might interplay with lipopolysaccharide to up-regulate MMP expression. Indeed, the present study clearly demonstrates that high glucose markedly augments lipopolysaccharide-stimulated MMP-1 expression by mononuclear phagocytes. To our best knowledge, this is the first report describing a synergistic effect between high glucose and lipopolysaccharide on MMP-1 expression. This finding may explain why periodontal tissue destruction in diabetic patients is more severe than that in non-diabetic individuals.

Another interesting finding from the present study is that prolonged pre-exposure of U937 cells to high glucose is required for enhancement of lipopolysaccharide-stimulated MMP-1 expression. Our data show that the pre-exposure to high glucose for a period of at least 2 weeks is necessary for the enhancement of lipopolysaccharide-stimulated MMP-1 expression. Clearly, a chronic effect of high glucose is involved in the augmentation. A similar observation was made previously by Tsao *et al.*, who reported that acute (24-h) incubation with high glucose had no effect on MMP-9 secretion by bovine aortic endothelial cells, whereas chronic (2-week) incubation with high glucose dramatically increased MMP-9 secretion (24). Although these authors did not further challenge the cells with inflammation mediators such as lipopolysaccharide, their study did demonstrate that a chronic effect of high glucose is involved in MMP expression.

High glucose has been shown to have both acute and chronic effects on signal activation and gene expression. For examples, Kanwar *et al.* reported that treatment of renal glomerular mesangial cells with high glucose for 24 h led to stimulation of fibronectin expression, and the stimulation was mediated by the protein kinase C signaling pathway (25). More interestingly, Hattori *et al.* reported that the peak stimulation of plasminogen activator-1 expression by high glucose was as short as 4 h and the stimulation was triggered by MAPK and protein kinase C activation (26). On the other hand, a large number of studies have shown that high glucose has chronic effect on gene expression and signaling activities. For instance, Whiteside *et al.* showed that only after

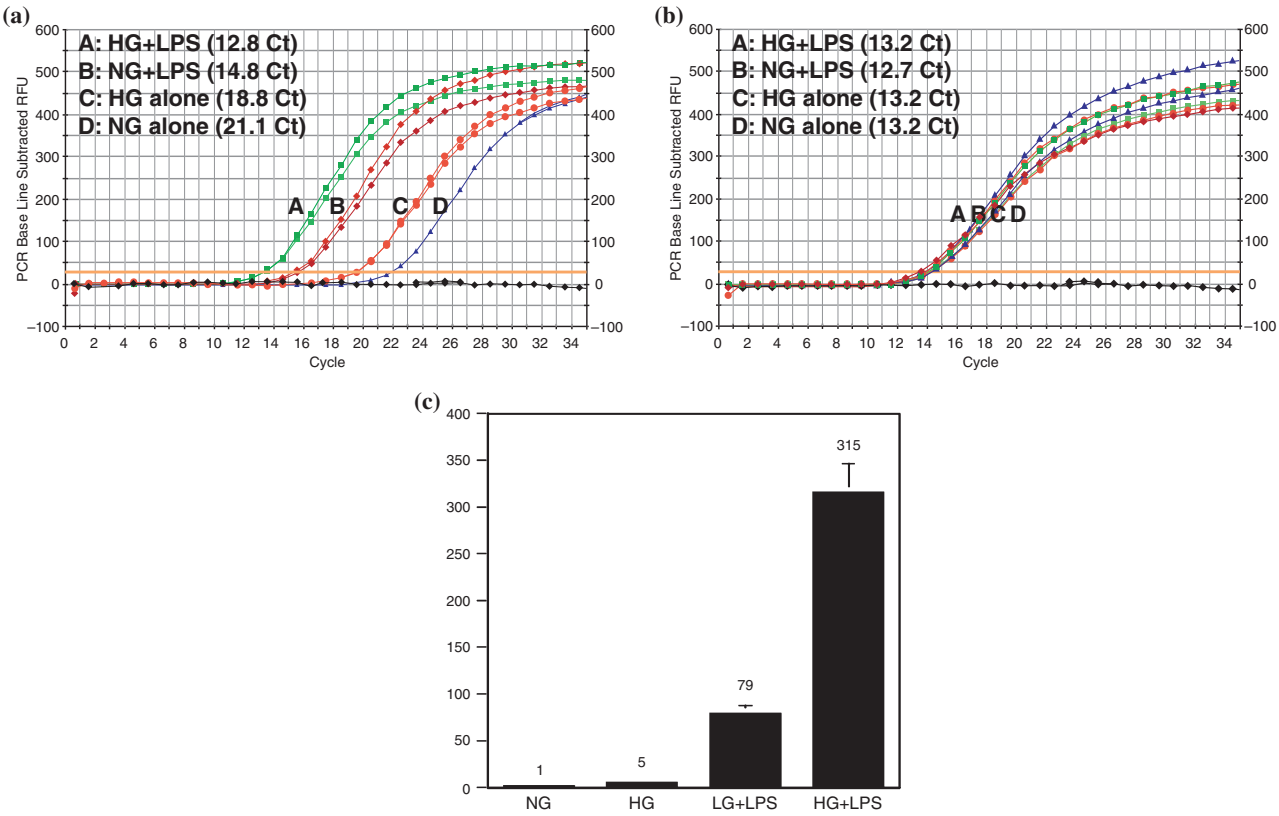


*Fig. 5.* Quantitative real-time polymerase chain reaction (PCR) analysis of cellular matrix metalloproteinase-1 (MMP-1) mRNA level. U937 cells were pre-exposed to normal glucose (NG) or high glucose (HG) for 4 weeks and then treated with 100 ng/ml of lipopolysaccharide (LPS) for 24 h. Total RNA was isolated and 5 μg of RNA was converted to cDNA by reverse transcription. The real-time PCR was performed with duplicate cDNA samples as described in Methods. The curves represent the real-time amplification of MMP-1 cDNA (A) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (B). The number of cycles before the amount of amplified cDNA reaches to the threshold is designated as $C_t$ (threshold cycles). The amount of PCR products is indicated by relative fluorescence units (RFU, *y*-axis). The data were processed and presented as fold increase of control (the amount of MMP-1 mRNA in normal glucose-exposed cells without LPS treatment) (C). The numbers above the bars indicate the fold of control.

treatment of rat mesangial cells with high glucose for 3 days, the phosphorylated p38 MAPK level was found to be significantly increased as compared to that in cells treated with normal glucose (27). A recent study by Natarajan *et al.* demonstrated that human monocytic THP-1 cells, after exposure to high glucose for 3 days, had increased expression of proinflammatory cytokine and chemokine genes (28). Tsao and coworkers showed that a 2-week incubation of vascular endothelial cells with high glucose increased MMP-9 expression (24). Thus, *in vitro* studies have demonstrated both acute and chronic effects of high glucose on signaling activation and gene expression. It should be noted, however, that the effects of hyperglycemia on gene expression *in vivo* are most likely to be chronic, as diabetes is a chronic disease, and cells, tissues, and organs are exposed to high glucose for months and years. It is possible that some patients may have had long-standing

*Table 2.* The effect of high glucose on matrix metalloproteinase (MMP) expression and on lipopolysaccharide (LPS)-stimulated MMP expression

| MMP | NG | HG | NG + LPS | HG + LPS | HG + LPS/NG + LPS |
|---|---|---|---|---|---|
| MMP-7 | 1 | 17 | 13 | 45 | 3.5 |
| MMP-8 | 1 | 11 | 108 | 194 | 1.8 |
| MMP-9 | 1 | 9 | 13 | 42 | 3.2 |

NG, normal glucose; HG, high glucose.
U937 cells were pre-exposed to normal (5 mM) or high glucose (25 mM) for 4 weeks and then treated with 100 ng/ml of LPS for 24 h. RNA was isolated from cells after the treatment and MMP mRNA was analyzed by DNA array hybridization as described in Methods. The amount of MMP mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The amount of MMP mRNA in cells treated with normal glucose alone is designated as 1 (control) and other values are presented as fold of control.

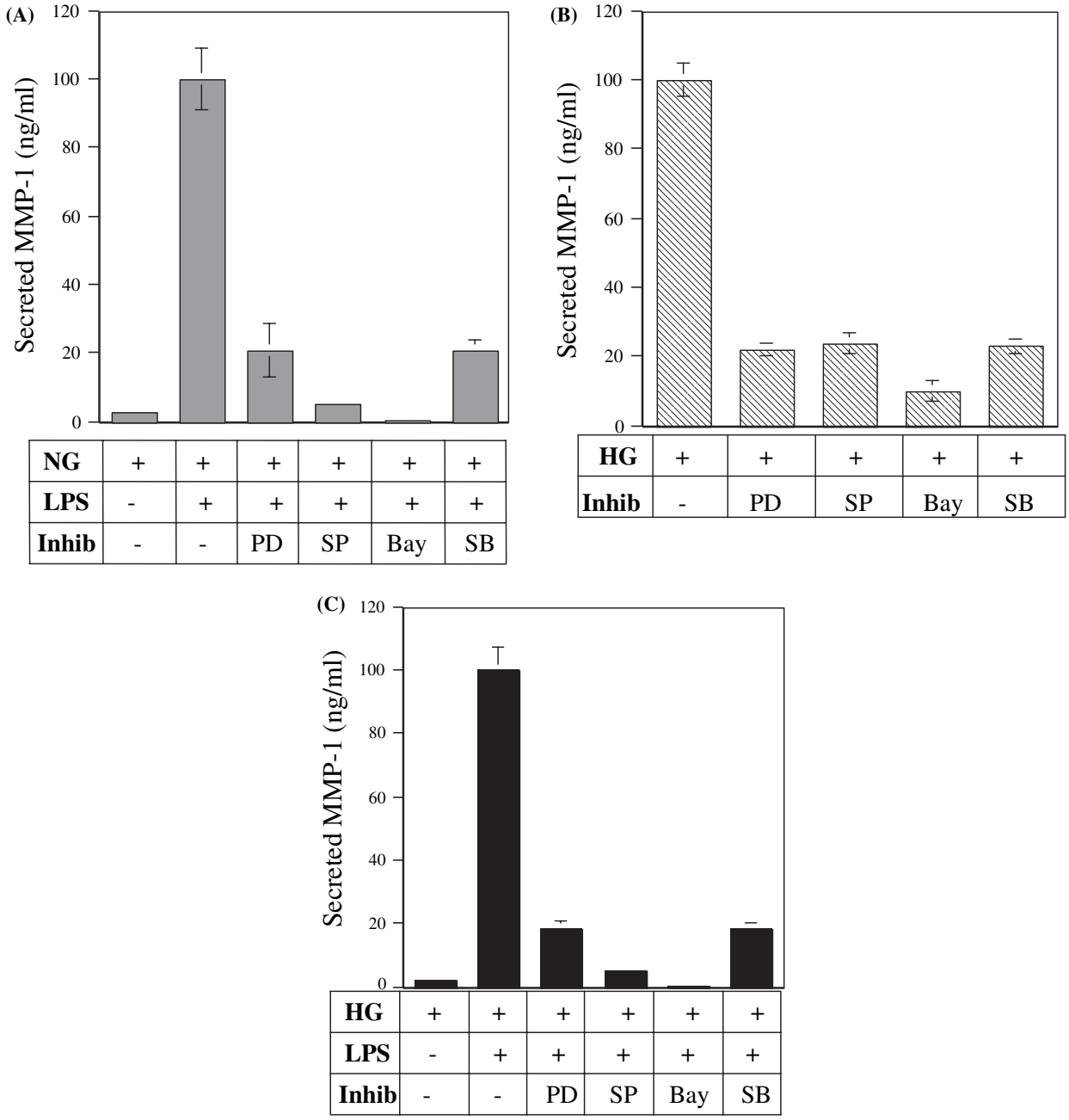


*Fig. 6.* The inhibition of matrix metalloproteinase (MMP) secretion from cells treated with lipopolysaccharide (LPS) (A), high glucose (B), or both (C) by specific inhibitors. (A) U937 cells were pre-exposed to normal glucose (NG, 5 mM) for 4 weeks and then treated with LPS (100 ng/ml) in the presence or absence of 10 μM of PD98059 (PD), SP600125 (SP), Bay 11-7085 (Bay), or SB203580 (SB) for 24 h. (B) U937 cells cultured with high glucose-containing medium (HG, 25 mM) for 4 weeks were exposed to fresh high glucose-containing medium in the absence or presence of 10 μM of PD98059, SP600125, Bay 11-7085, or SB203580 for 24 h. (C) U937 cells pre-exposed to high glucose (25 mM) for 4 weeks were treated with LPS (100 ng/ml) in the presence or absence of 10 μM of PD98059, SP600125, Bay 11-7085, or SB203580 for 24 h. After the treatment, secreted MMP-1 in the conditioned medium was quantified by ELISA.

exposure to lipopolysaccharide and high glucose levels only become evident later in life. Since lipopolysaccharide is a potent stimulator of MMP-1, it would increase MMP-1 expression before hyperglycemia occurs. However, high glucose that becomes evident later in these patients is likely to augment lipopolysaccharide-induced MMP-1 expression when they are exposed to lipopolysaccharide after having hyperglycemia.

It has been reported previously that lipopolysaccharide up-regulates MMP-1 expression in monocytes through ERK1/2 and p38 activation (29). Our present study showed that, in addition to ERK1/2 and p38 cascades, JNK and

NFκB pathways were also involved. Thus, it appears that lipopolysaccharide stimulates MMP-1 expression through multiple signaling pathways, which is consistent with the previous reports that lipopolysaccharide activates NFκB and MAPK pathways (10). In our studies to explore the mechanisms by which high glucose augments lipopolysaccharide-stimulated MMP-1 expression, we found that the same signaling pathways such as NFκB, ERK, JNK, and p38 cascades were also involved in high glucose-stimulated MMP-1 expression (Fig. 6B). Thus, it is likely that high glucose increases lipopolysaccharide-stimulated MMP-1 expression by enhancing lipopolysaccharide-elicited signaling through the NFκB and MAPK pathways. It would be interesting to find out how these pathways coordinate for MMP-1 gene expression. Thus, more experiments are warranted for further investigation on the signaling and molecular mechanisms.

In conclusion, the present study demonstrates that pre-exposure of mononuclear phagocytes with high glucose augments lipopolysaccharide-stimulated MMP-1 expression. This finding suggests that hyperglycemia in diabetes may promote periodontal disease by increasing susceptibility of mononuclear phagocytes to bacterial pathogens and thus enhancing the degradation of periodontal tissue.

## Acknowledgements

This work was supported by a Merit Review Grant from the Research Service of the Department of Veterans Affairs and an institutional grant from Medical University of South Carolina.

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