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Metabolism Clinical and Experimental 57 (2008) 1725–1732
Metabolism
Clinical and Experimental
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High glucose induces transactivation of the human paraoxonase 1 gene in hepatocytes

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Received 11 January 2008; accepted 1 July 2008

Abstract

Human serum paraoxonase 1 (PON1) is associated with high-density lipoprotein and inhibits oxidative modification of low-density lipoprotein in vitro. Therefore, PON1 is expected to protect against atherosclerosis in vivo. We and other investigators have shown that PON1 enzymatic activity is decreased in diabetic patients; however, an alteration in hepatic PON1 synthesis under hyperglycemic conditions remains unclear. We previously demonstrated that Sp1 is a positive regulator of PON1 transcription and that an interaction between Sp1 and protein kinase C (PKC) is a crucial mechanism for the effect of Sp1 on PON1 transcription in cultured HepG2 cells. Because several PKC isoforms are activated under hyperglycemic conditions, we examined the effect of D-glucose, which can activate the diacylglycerol-PKC pathway, on the transcription and expression of PON1. For a reporter gene assay, Huh7 human hepatocyte cell line incorporated with PON1 (−1232/−6)–luciferase expression vector was established using a cationic lipid method. D-Glucose dose dependently enhanced PON1 promoter activity. D-Glucose also enhanced both messenger RNA and protein expression of PON1. Increased PON1 expression was also detected in primary human hepatocytes treated with high D-glucose concentrations. Bisindolylmaleimide, a PKC inhibitor, significantly inhibited D-glucose–induced transactivation of PON1; and mithramycin, an inhibitor of Sp1, completely abrogated the transactivation. Our data suggest that high glucose concentrations transactivate the PON1 gene through Sp1 activation by PKC in cultured hepatocytes. Up-regulated hepatic PON1 expression under high glucose conditions may be a compensatory mechanism in diabetes in which antioxidant capacity, including PON1 enzymatic activity, is attenuated.

1. Introduction

The oxidative modification of low-density lipoprotein (LDL) plays a central role in the initiation and acceleration of atherosclerosis [1,2]. Oxidized LDL exerts several proatherogenic effects, including increased synthesis and secretion of adhesion molecules, monocyte chemotaxis and adhesion, platelet adhesion and aggregation, coagulation, vasoconstriction, apoptotic effects on endothelial cells, enhanced foam cell formation, and increased smooth muscle cell migration and proliferation [3-5]. Both increased oxidative stress and decreased antioxidant capacity can promote lipid peroxidation. The inverse correlation between high-density lipoprotein (HDL) cholesterol and risk of cardiovascular disease is well established, and a critical function of HDL is to mediate the efflux of cholesterol from vascular macrophages and other peripheral tissues [6,7]. In addition to the role in reverse cholesterol transport, HDL also has antioxidant, anti-inflammatory, and antithrombotic properties [8-10].

Human serum paraoxonase 1 (PON1), which is an esterase associated with HDL [11], reduces the susceptibility of LDL to lipid peroxidation in vitro [12,13]; therefore, this enzyme is thought to play a central role in the inhibitory effect of HDL on lipid peroxidation. In fact, PON1 knockout mice have been shown to be susceptible to the development of atherosclerosis [14,15]. In accordance with these observations, transgenic overexpression of PON1 in mice was shown to result in decreased atherosclerotic lesion formation [16,17]. These data support a physiologic role of PON1 in reducing the susceptibility to lipid peroxidation, which has been previously described in a number of in vitro studies. The PON1 gene, as well as the gene family PON2 and PON3, is located on chromosome 7q21.3-q22.1; and the liver plays a key role in PON1 synthesis [18].

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0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.07.032

Considerable evidence suggests that hyperglycemia is associated with excessive oxidation [19,20]. Defective antioxidant capacity including deficient HDL function may be 1 feature of the greater susceptibility to oxidation in diabetes. In fact, HDL from subjects with type 2 diabetes mellitus was shown to have a decreased ability to metabolize oxidized phospholipids [21]. Several previous studies have shown that enzymatic activities of HDL-associated PON1 were decreased in patients with type 1 diabetes mellitus [22-24] and in those with type 2 diabetes mellitus [23,25-27], as well as in streptozotocin-diabetic rats [28]. These decreases in enzymatic activity may be explained by glycation [29,30] and oxidative inactivation [31,32]. Serum PON1 concentration has also been shown to be lower in diabetic patients than in healthy controls [24,26], although some studies failed to detect a significant difference [23,27]. This could also be explained partly by glycation or compositional changes of HDL in the diabetic state because it has been shown that glycation of HDL apolipoproteins can enhance their catabolism [33]. However, besides accumulating data regarding glycation or oxidative modification of PON1, an alteration in the hepatic PON1 synthesis in the diabetic state remains unclear. We have previously demonstrated that Sp1 is a positive regulator of PON1 transcription and that Sp1 activation by protein kinase C (PKC) is a crucial mechanism for the effect of Sp1 on PON1 transcription in cultured HepG2 cells [34]. Because, under hyperglycemic conditions, several PKC isoforms are activated by increased diacylglycerol (DAG) content [35,36], the high glucose concentration may induce transactivation of the PON1 gene. Our study aimed to clarify the effects of high glucose concentrations on the transcription and expression of PON1 and its mechanisms of action in human hepatocyte cell lines.

2. Materials and methods

2.1. Materials

Dulbecco modified Eagle medium, penicillin, streptomycin, and fetal calf serum were obtained from Invitrogen (Carlsbad, CA); and all tissue culture plastic wares were from Nunc (Roskilde, Denmark). The PKC inhibitor bisindolylmaleimide (BIM) was from Calbiochem (La Jolla, CA). Protein assay reagents were obtained from Bio-Rad (Hercules, CA); and nitrocellulose membrane, from Amersham Biosciences (Buckinghamshire, United Kingdom). The Sp1 inhibitor mithramycin and all other chemicals were obtained from Sigma (St Louis, MO).

Polyclonal rabbit antibodies against PON1 were prepared in our laboratory as previously described [27]. Horseradish-peroxidase–conjugated secondary antibodies were obtained from ICN Pharmaceuticals (Aurora, OH).

2.2. Complementary DNAs

For the reporter gene assay, a DNA fragment of the promoter region of the PON1 gene (−1232/−6) was amplified by polymerase chain reaction (PCR) using a sense primer: 5′-*GGGGTACC*CTCTCCATATGTTCATGG-3′ and an antisense primer: 5′-*TCCCCCGGG*ATAGACAAAGGGATCG-3′. The underlined area indicates the sequence added as restriction sites of *Kpn*I and *Sma*I, respectively. For deletion analysis, various lengths of plasmids including −587/−6, −269/−6, and −97/−6 were generated using the same antisense primer and sense primers: 5′-GG*GGTACC*AGCTGCATGAGGAAATG-3′, 5′-GG*GGTACC*TGGACTAGGCACCTATTCTC-3′, and 5′-GG*GGTACC*AATCGGCGCTGCCCCAGC-3′, respectively. Although there were 3 single nucleotide polymorphisms in the PON1 promoter region, the template genomic DNA used for the reporter gene assay was obtained from the −108CC genotype whose −126 and −160 bases were guanine. The DNA fragment was introduced into the firefly luciferase expression vector pGL3-Basic (Promega, Madison, WI) and subcloned into JM109 bacterial cells. The introduced fragments were confirmed by cycle sequencing methods.

2.3. Cell culture

Huh7 and HepG2 human hepatoma cells and human embryonic kidney (HEK) 293 cells were cultured and maintained in Dulbecco modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 20 μg/mL streptomycin. Primary human hepatocytes CC2591 (14 years old, female) from Cambrex Bio Science (Walkersville, MD) were used for Western blot analysis. Cryopreserved human hepatocytes were quickly thawed in a 37°C water bath with gentle shaking. Afterward, the cell suspension was transferred slowly into a tube containing 25 mL of cold hepatocyte culture medium (HCM, Cambrex Bio Science) and centrifuged at 50*g* at 4°C for 3 minutes. The HCM is constituted from hepatocyte basal medium together with all components provided in the HCM Bulletkit (Cambrex Bio Science): hepatocyte growth factors, insulin, ascorbic acid, transferrin, bovine serum albumin (fat-acid free), gentamicin 50 μg/mL, and amphotericin B 50 ng/mL. The cell pellet was suspended in HCM. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

2.4. Transfection, treatment, and reporter gene assay

For transient transfection experiments, cells were seeded in 24-well plates; and the test plasmids were transfected with Tfx-20 transfection reagents (Promega) for Huh7 and HepG2 cells and with TransFast transfection reagents (Promega) for HEK293 cells. The pRL-TK vector, which expressed Renilla luciferase (Promega), was cotransfected as an internal control for transfection efficiency. The final transfected DNA amount in each well was adjusted by the addition of empty vectors. Twenty-four hours after transfection, the culture medium was replaced with serum-free and glucose-free medium; and the cells were then treated with the test substances for a defined period. We used BIM as a classic and novel PKC inhibitor and mithramycin as an Sp1

inhibitor at final concentrations of 1 μmol/L for BIM and 100 nmol/L for mithramycin. When both D-glucose and BIM or mithramycin were used, the cells were pretreated with BIM or mithramycin; and D-glucose was then added 60 minutes later. Both luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega), and results were expressed as a ratio of firefly luciferase activity to Renilla luciferase activity.

For stable transfection experiments, a mixed polyclonal Huh7 cell line incorporated with the PON1 (−1232/−6)–luciferase expression vector, designated as *Huh7-PON*, was established with antibiotic G-418 sulfate (Promega) according to the manufacturer's instructions. Firefly luciferase activity was measured using the Luciferase Reporter Assay System (Promega), and results were normalized to the protein content of cell lysates.

2.5. Real-time PCR

Total RNA was extracted using a commercial kit (RNeasy Mini kit; Qiagen, Tokyo, Japan). Reverse transcription was performed using a commercial kit including TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA) with random hexamers as primers. Target amplification using complementary DNA as a template was performed with TaqMan Universal Master Mix and TaqMan Gene Expression Assay reagents including the conditioned primer and probe for human PON1 gene (*Hs00166557_m1*, Applied Biosystems) according to the manufacturer's instructions. As an endogenous control, a TaqMan probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Applied Biosystems) was also used to detect messenger RNA (mRNA) expression of a housekeeping gene. Polymerase chain reaction amplification involved 40 cycles, as follows: 15 seconds at 95°C for denaturation and 1 minute at 60°C for annealing and extension. Reactions were amplified and quantified using an ABI PRISM 7000 sequence detector and the manufacturer's corresponding software.

2.6. Gel electrophoresis and Western blot analysis

The cells were lysed in 300-μL/3.5-cm dish using buffer I (20 mmol/L Tris acetate [pH 7.0], 0.27 mol/L sucrose, 1 mmol/L EGTA, 1 mmol/L EDTA, 1% Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L benzamidin, and 5 μg/μL leupeptin). Supernatants were obtained by centrifugation at 15 000 rpm for 10 minutes at 4°C. The protein concentration was normalized (Bio-Rad Protein Assay), and samples (10 μg protein per sample) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. After separation, the proteins were transferred to a nitrocellulose membrane, which had been blocked in 1× phosphate-buffered saline (PBS) buffer containing 0.2% Tween 20 (PBS-Tween buffer) and 3% milk powder and subsequently in 1× PBS-Tween buffer containing 0.2% gelatin before overnight incubation with polyclonal antibodies for PON1. Immunoreactive proteins were visualized using horseradish-peroxidase–coupled secondary antibodies and enhanced chemiluminescence (ECL) reagents.

2.7. Data analyses

Each experiment was repeated a least 3 times. Results of the reporter gene assay are presented as means ± SEM. For statistical analyses, data were compared by 1-way analysis of variance with a Fisher protected least significant difference test. *P* values less than .05 were considered significant.

3. Results

3.1. Effects of high glucose on PON1 promoter activity in Huh7-PON

To examine the effects of D-glucose on PON1 transcription, Huh7-PON were treated with 5 to 30 mmol/L D-glucose. As shown in Fig. 1, D-glucose dose dependently (from 5 to 20 mmol/L) enhanced the PON1 promoter activity; and the promoter activity was then sustained at up to 25 mmol/L. The highest promoter activity was observed when the cells were incubated with 25 mmol/L D-glucose for 12 to 48 hours (Fig. 2). Therefore, in later experiments, we treated cells with 25 mmol/L D-glucose for 24 hours to investigate the effects of D-glucose on PON1 promoter activity. Both L-glucose and mannitol had no effect on PON1 promoter activity (Fig. 3).

3.2. Effects of high glucose on PON1 promoter activity in various cell lines

To examine whether D-glucose–induced transactivation of the PON1 gene could be detected in other cell lines, we carried out transient transfection experiments using human hepatoma

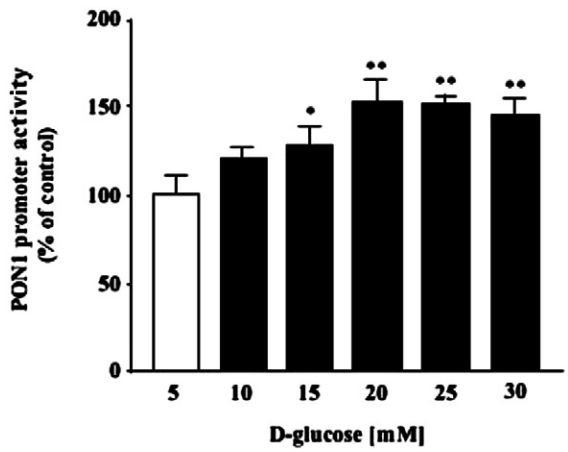

Fig. 1. Dose-dependent effects of D-glucose on PON1 promoter activity in Huh7-PON hepatocytes. The reporter gene assay was performed in Huh7-PON hepatocytes. Final D-glucose concentrations are indicated (5-30 mmol/L). Firefly luciferase activity and protein content were assayed as described in "Materials and methods." Paraoxonase 1 promoter activity is expressed as firefly luciferase activity/protein content. Basal promoter activity (white bar) is set as 100%, and relative activities are presented. Data represent means ± SEM (n = 4). **P* < .05, ***P* < .001 vs basal promoter activity.

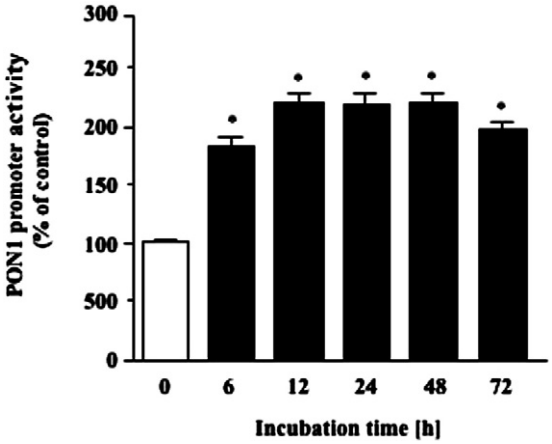

Fig. 2. Time-course effects of high glucose concentrations on PON1 promoter activity in Huh7-PON hepatocytes. The reporter gene assay was performed in Huh7-PON hepatocytes. Treatments with 25 mmol/L D-glucose for specific times were performed as indicated. Firefly luciferase activity and protein content were assayed as described in "Materials and methods." Paraoxonase 1 promoter activity is expressed as firefly luciferase activity/protein content. Basal promoter activity (white bar) is set as 100%, and relative activities are presented. Data represent means ± SEM (n = 4). *$P < .0001$ vs basal promoter activity.

cell lines Huh7 and HepG2 and human embryonic kidney cell line HEK293. As shown in Fig. 4, D-glucose dose dependently enhanced the PON1 promoter activity in all the cell types.

3.3. Effects of high glucose on PON1 mRNA and protein expressions in Huh7 cells

To investigate whether D-glucose–induced transactivation leads to the enhancement of PON1 expression, we carried out real-time (RT)–PCR and Western blot analysis. In RT-PCR, treatment with 25 mmol/L D-glucose significantly increased PON1 mRNA expression in Huh7 cells (Fig. 5A). Western blot analysis showed that D-glucose treatment (25 mmol/L, 24 hours) also enhanced PON1 protein expression (Fig. 5B).

3.4. Effects of high glucose on PON1 in primary human hepatocytes

We then examined whether D-glucose up-regulates PON1 in normal human hepatocytes because both Huh7 and HepG2 are hepatoma cell lines. Western blot analysis clearly demonstrated that treatment with 25 mmol/L D-glucose significantly increased PON1 expression in primary human hepatocytes (Fig. 6).

3.5. Roles of PKC and Sp1 on high glucose–induced transactivation of the PON1 gene

Because hyperglycemia activates the DAG-PKC pathway and we have shown that Sp1 activation by PKC is a crucial mechanism in transcriptional regulation of the PON1 gene [34], we assumed that Sp1 and PKC are involved in D-glucose–induced transactivation.

First, we carried out deletion analysis to identify which region in the PON1 upstream sequence was crucial for glucose-induced transactivation (Fig. 7). Fragments −1232/−6, −587/−6, and −269/−6 contained the Sp1 site; and fragment −97/−6 contained no Sp1 site. Fragments −1232/−6, −587/−6, and −269/−6 showed almost equal responses to high glucose concentrations. However, fragment −97/−6, which lacks the Sp1 site, showed markedly reduced basal promoter activity and did not respond to high glucose conditions.

We next examined the effects of PKC and Sp1 inhibition (Fig. 8). Bisindolylmaleimide partially but significantly inhibited D-glucose–induced transactivation of the PON1 gene, and mithramycin completely abrogated the transactivation.

4. Discussion

Previous studies have demonstrated that LDL oxidation is enhanced by high concentrations of glucose [37,38]. Hyperglycemia is also associated with mild but chronic inflammation, which can accelerate the oxidation of lipoproteins [39]. Furthermore, nonenzymatic glycation of LDL makes it more susceptible to oxidation [40]. Therefore, excessive oxidation of lipoproteins, which results from hyperglycemia, seems to be a crucial mechanism for the high susceptibility of atherosclerosis in diabetes. Although a number of studies have shown that enzymatic activities of HDL-associated antioxidant PON1 were decreased in diabetic patients [22-27], an alteration in hepatic PON1 expression under hyperglycemic condition remained unclear, which prompted us to study the effect of

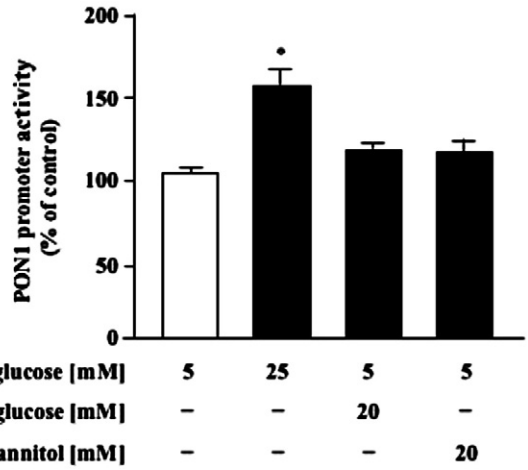

Fig. 3. Effects of L-glucose and mannitol on PON1 promoter activity in Huh7-PON hepatocytes. The reporter gene assay was performed in Huh7-PON hepatocytes. Final concentrations of D-glucose, L-glucose, and mannitol are indicated. Firefly luciferase activity and protein content were assayed as described in "Materials and methods." Paraoxonase 1 promoter activity is expressed as firefly luciferase activity/protein content. Basal promoter activity (white bar) is set as 100%, and relative activities are presented. Data represent means ± SEM (n = 6). *$P < .001$ vs basal promoter activity.

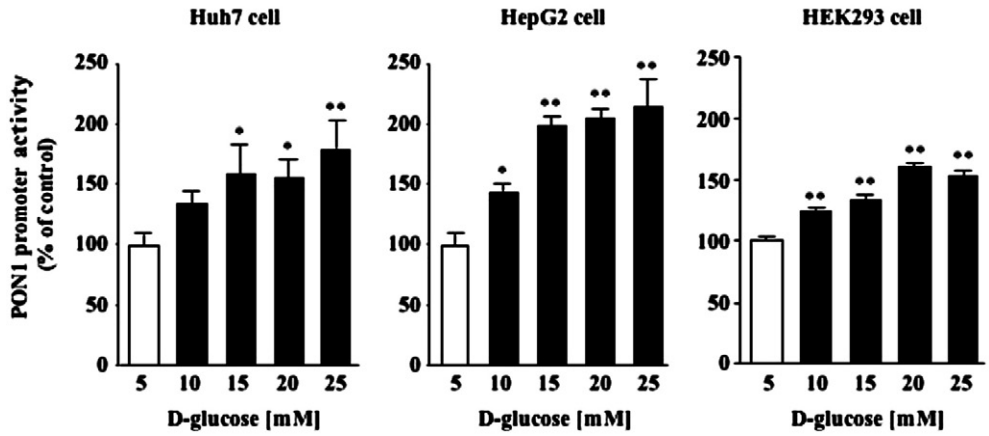

Fig. 4. Effects of high glucose concentrations on PON1 promoter activity in Huh7, HepG2, and HEK293 cells. The reporter gene assay was performed in Huh7, HepG2, and HEK293 cells. Cells were seeded into 24-well dishes and were transiently transfected with pGL3-Basic containing the PON1 promoter region (−1232/−6) and cotransfected with pRL-TK as an internal control. Treatment with D-glucose was started 24 hours after transfection, and final concentrations are indicated (5-25 mmol/L). Firefly and Renilla luciferase activities were assayed as described in "Materials and methods." Paraoxonase 1 promoter activity is expressed as firefly luciferase activity/Renilla luciferase activity. Each basal promoter activity (white bar) is set as 100%, and relative activities are presented. Data represent means ± SEM (n = 4). *$P < .05$, **$P < .01$ vs basal promoter activity.

glucose on transcription and expression of PON1 in cultured hepatocytes.

In this study, we demonstrated that high glucose concentrations dose dependently enhanced the transcription and expression of PON1. Because L-glucose and mannitol had no effect on PON1 promoter activity, the effect of D-glucose on promoter activity was considered to be through its metabolic actions. We found that this up-regulatory effect of glucose on PON1 transcription is detected not only in Huh7 cells but also in HepG2 and HEK293 cells. Furthermore, we confirmed that this transactivation leads to increased PON1 expression in both Huh7 cells and primary human hepatocytes.

The sequence immediately upstream of the PON1 gene contains no TATA box; and the region is a GC-rich, typical

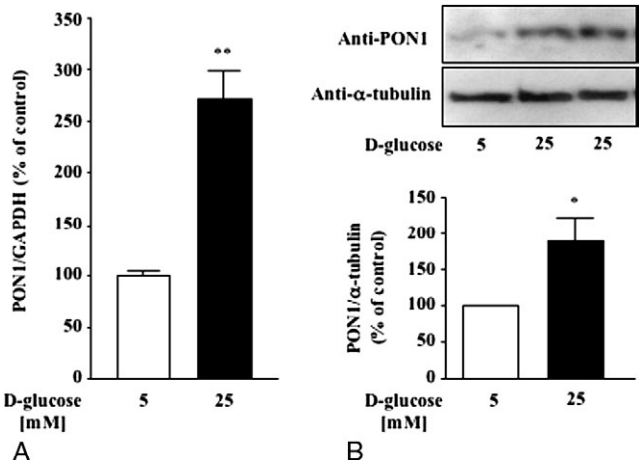

Fig. 5. Effects of high glucose concentrations on mRNA and protein expressions of PON1 in Huh7 cells. A, Huh7 cells were seeded into 24-well dishes and treated with 5 or 25 mmol/L D-glucose for 24 hours. Total RNA was extracted using a commercial kit, and mRNA expression levels of PON1 and GAPDH were determined using RT-PCR as described in "Materials and methods." Messenger RNA level is expressed as that of PON1 divided by GAPDH as endogenous control (PON1/GAPDH). Basal mRNA expression with 5 mmol/L D-glucose (white bar) is set as 100%, and relative expressions are presented. Data represent means ± SEM (n = 6). **$P < .0001$ vs basal PON1/GAPDH. B, Huh7 cells were seeded into 6-well dishes and treated with 5 or 25 mmol/L D-glucose for 24 hours. The expression of PON1 protein was detected by Western blot analysis using antibodies specific for PON1. The immunoreactive proteins were visualized using horseradish-peroxidase–coupled secondary antibodies and the ECL detection method. Data represent means ± SEM (n = 5). *$P < .05$ vs basal PON1/α-tubulin.

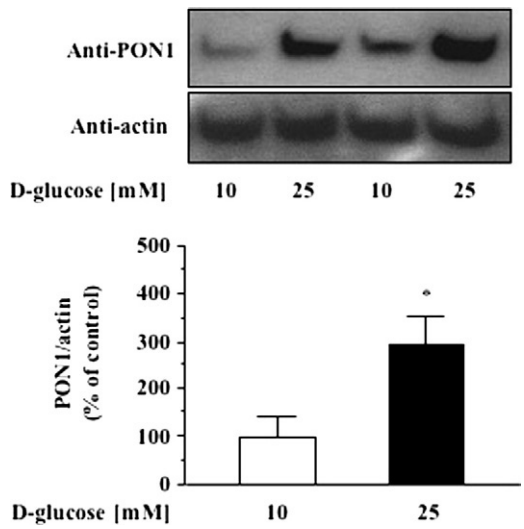

Fig. 6. Effects of high glucose concentration on PON1 protein expression in primary human hepatocytes. Primary human hepatocytes were seeded into 6-well dishes and treated with 10 or 25 mmol/L D-glucose for 24 hours. The expression of PON1 protein was detected by Western blot analysis using antibodies specific for PON1. The immunoreactive proteins were visualized using horseradish-peroxidase–coupled secondary antibodies and the ECL detection method. *$P < .05$ vs basal PON1/actin (n = 5).

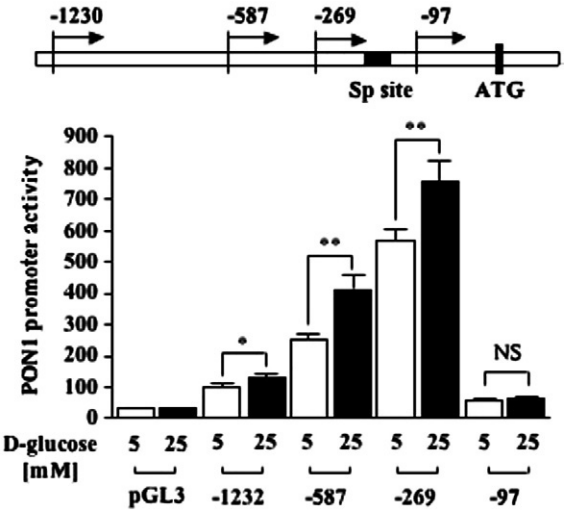

Fig. 7. Deletion analysis of the 5′-upstream region of the PON1 gene in Huh7 cells. The reporter gene assay was performed in Huh7 cells using pGL3-Basic vectors containing various lengths of the PON1 promoter region. The methods for the generation of plasmids, transient transfections, and reporter gene assay are described in "Materials and methods." Treatment with D-glucose was started 24 hours after transfection. Firefly and Renilla luciferase activities were assayed as described in "Materials and methods." Paraoxonase 1 promoter activity is expressed as firefly luciferase activity/Renilla luciferase activity. Mean promoter activity of the −1232/−6 fragment with 5 mmol/L D-glucose is set as 100%, and relative activities to the −1232/−6 fragment are presented. The white bars show the activities in cells incubated with 5 mmol/L D-glucose, and the black bars show the activities in cells incubated with 25 mmol/L D-glucose. Data represent means ± SEM (n = 4). The locations of potential binding sites for Sp1 are shown (upper panels). *$P < .05$, **$P < .001$. NS indicates not significant.

TATA-less promoter. Sp1, which is a ubiquitous mammalian transcription factor belonging to the C2-H2 zinc finger family, binds to GC-rich nucleotide sequences (GC boxes) and activates transcription of many different cellular genes. We have previously shown the binding of Sp1 to the PON1 upstream sequence (−125/−95) and Sp1-induced transactivation of the PON1 gene using an electrophoretic mobility shift analysis and a reporter gene assay, respectively [34]. Sp1 is thought to be required for the glucose-responsive transactivation of several genes such as transforming growth factor−α and plasminogen activator inhibitor−1 [41-43]. We therefore tested the hypothesis that Sp1 is also involved in glucose-induced transactivation of the PON1 gene. We showed that Sp1 inhibition with mithramycin completely abrogated glucose-induced transactivation; therefore, the effects of glucose were considered to occur through Sp1 activation. In accordance with this, in a deletion analysis, the promoter fragment lacking the Sp1 site showed not only reduced basal activity but also unresponsiveness to high glucose conditions.

Protein phosphorylation is one of the major mechanisms regulating transcription factor activity by affecting DNA binding either positively or negatively. We previously demonstrated that transactivation of the PON1 gene induced by phorbol 12-myristate 13-acetate, a PKC activator, was inhibited by mithramycin, suggesting that Sp1 activation by PKC is a crucial mechanism for PON1 gene transactivation [34]. Therefore, it was of interest to investigate whether PKC is involved in the glucose-induced transactivation of the PON1 gene because hyperglycemia activates the DAG-PKC pathway. As expected, we found that the PKC inhibitor BIM significantly inhibited the transactivation.

Because, in diabetes, conventional and novel PKCs in various tissues including the liver are supposed to be activated by accelerated de novo synthesis of DAG, our idea that PKC activation up-regulates PON1 seems to be inconsistent with previous in vivo data showing decreased

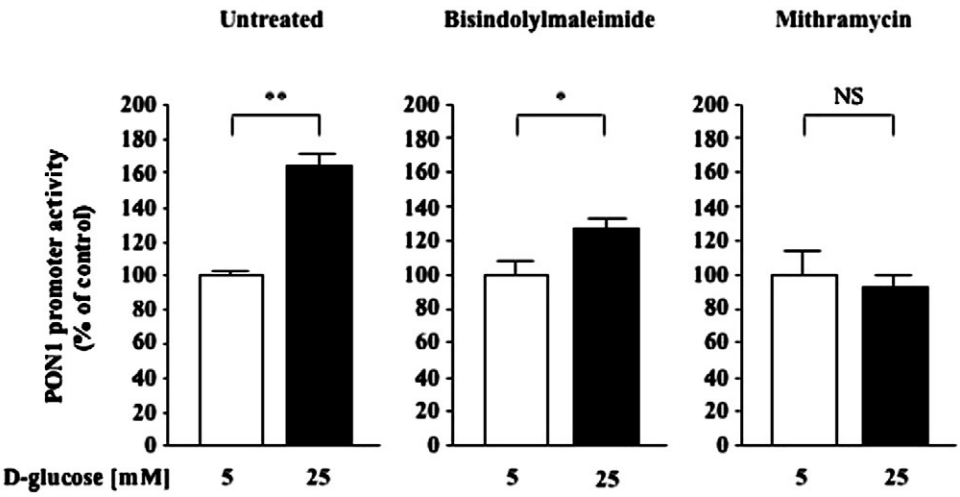

Fig. 8. Effects of inhibition of PKC and Sp1 on glucose-induced transactivation of the PON1 gene in Huh7-PON hepatocytes. The reporter gene assay was performed in Huh7-PON hepatocytes. The cells were pretreated with 1 μmol/L BIM or 100 nmol/L mithramycin, and D-glucose was then added another 60 minutes later. Firefly luciferase activity and protein content were assayed as described in "Materials and methods." Paraoxonase 1 promoter activity is expressed as firefly luciferase activity/protein content. Each basal promoter activity with 5 mmol/L D-glucose (white bar) is set as 100%, and relative activities with 25 mmol/L D-glucose (black bars) are presented. Data represent means ± SEM (n = 6). *$P < .05$, **$P < .0001$.

serum PON1 concentrations in diabetic patients [24,26]. If our results are applicable to the human liver, decreased PON1 in diabetes may be due to the modification of circulating HDL or PON1 per se in diabetic states such as glycation and oxidative inactivation [29-33]. However, the transcriptional regulation in diabetes in vivo may be more complex because factors other than hyperglycemia (eg, hyper-/hypoinsulinemia, oxidative stress, microinflammation) could also influence PON1 transcription. In fact, we previously found that interleukin-1β and tumor necrosis factor–α repressed PON1 gene transcription in HepG2 cells [44]. In addition, we did not examine the long-term effect of high glucose concentrations on PON1 transcription/expression, which may be different from the short-term effect. Further in vivo work is required to clarify the regulation of hepatic PON1 synthesis in diabetes.

In summary, we have demonstrated for the first time that high glucose concentrations transactivated the PON1 gene and enhanced its expression through Sp1 activation by PKC in cultured hepatocytes. Up-regulated hepatic PON1 expression by glucose may be a compensatory mechanism in diabetes whose antioxidant capacity, including PON1 enzymatic activity, is attenuated.

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