Taurine prevents high-glucose-induced human vascular endothelial cell apoptosis

QIONG DI WU, JIANG HUAI WANG, FIONA FENNESSY, H. PAUL REDMOND, AND DAVID BOUCHIER-HAYES

The Royal College of Surgeons in Ireland, Department of Surgery, Beaumont Hospital, Dublin, Ireland

Wu, Qiong Di, J iang Huai Wang, Fiona Fennessy, H. Paul Redmond, and David Bouchier-Hayes. Taurine prevents high-glucose-induced human vascular endothelial cell apoptosis. Am. J. Physiol. 277 (Cell Physiol. 46): C1229–C1238, 1999.—Elevated blood glucose in uncontrolled diabetes is causally correlated with diabetic microangiopathy. Hyperglycemia-triggered accelerated endothelial cell apoptosis is a critical event in the process of diabetes-associated microvascular disease. The conditionally semiessential amino acid taurine has been previously shown to protect against human endothelial cell apoptosis. Therefore, this study was designed to investigate the role of taurine in the prevention of hyperglycemia-mediated cell apoptosis in human umbilical vein endothelial cells (HUVEC) and the mechanisms involved. Exposure of HUVEC to 30 mM glucose for 48 h (short-term) and 14 days (long-term) resulted in a significant increase in apoptosis, compared with normal glucose (5.5 mM; P < 0.05). High-glucose-induced DNA fragmentation preferentially occurred in the S phase cells. Mannitol (as osmotic control) at 30 mM failed to induce HUVEC apoptosis. Taurine prevented high-glucose-induced HUVEC apoptosis, which correlates with taurine attenuation of high-glucose-mediated increased intracellular reactive oxygen species (ROS) formation and elevated intracellular Ca2+ concentration ([Ca2+]i) level. Antioxidants, DMSO, N-acetyl cysteine, and glutathione, only partly attenuated high-glucose-induced HUVEC apoptosis. Glucose at 30 mM did not cause HUVEC necrosis. However, both glucose and mannitol at 60 mM caused HUVEC necrosis as represented by increased lactate dehydrogenase release and cell lysis. Taurine failed to prevent hyperosmolarity-induced cell necrosis. These results demonstrate that taurine attenuates hyperglycemia-induced HUVEC apoptosis through ROS inhibition and [Ca2+]i stabilization and suggest that taurine may exert a beneficial effect in preventing diabetes-associated microangiopathy.

bboth insulin-dependent and non-insulin-dependent diabetes mellitus are associated with an increased risk for atherosclerosis. Among the many metabolic abnormalities of the diabetic milieu, hyperglycemia is the one showing in epidemiological studies the most consistent and significant correlation with diabetic microangiopathy. Recently, the data from the Diabetes Control and Complication Trial and from the Eurodiab Insulin-Dependent Diabetes Mellitus Complications Study Group have both clearly implicated a causal relationship between poor control of blood glucose concentrations and diabetic microvascular disease (15, 25, 27).

The vascular lesions characteristic of human and experimental diabetes suggest a primary involvement of vascular endothelium. A growing body of evidence indicates the close correlation between hyperglycemia and the abnormalities in endothelial function and morphology. High ambient glucose has been reported to be toxic in vitro for endothelial cells, as represented by lengthened cell proliferation (14, 28, 30), disturbed cell cycle (30, 32), increased DNA damage (31), and slightly accelerated cell death (30). Exposure of endothelial cells to a high-glucose medium results in an impaired cellular radical scavenger function (5, 23). Moreover, high glucose seems to modulate adhesion molecule expression and enhance the adherence of both neutrophils and monocytes on endothelial cells (6, 9, 24). In vivo, hyperglycemia in diabetes have been found to be responsible for endothelial abnormalities (16, 26, 29), including accelerated disappearance of capillary endothelium and weakening of intracellular junctions.

Apoptosis or programmed cell death is an active, genetically controlled process of cell suicide, which is morphologically and biochemically different from necrosis (11). The cell undergoing apoptosis is characterized by cell shrinkage, nuclear condensation, and DNA fragmentation, whereas the cytoplasmic membrane remains intact during the early stage. Therefore, apoptosis, in contrast to necrosis, does not release its contents to damage surrounding tissues or provoke an inflammatory response. However, in the case of endothelial cells, we have previously demonstrated that induction of apoptosis leads to an alteration of endothelial barrier function resulted from the distortion of monolayer architecture as a consequence of the reduced size and altered shape of the apoptotic endothelial cells (41). Recently, two studies (an in vitro and an in vivo study) have demonstrated that high glucose selectively triggered apoptosis in cultured human endothelial cells (7) and that an accelerated programmed cell death of retinal microvascular cells occurs in situ in human and experimental diabetic retinopathy (35). These findings indicate that the occurrence of accelerated endothelial cell apoptosis is an important event in the process of diabetes-associated microangiopathy, including increased microvascular permeability and retinal capillary obliteration. Thus prevention of hyperglycemia-triggered endothelial cell apoptosis may have important implications for pharmacological attempts at preventing diabetes-associated microvascular complications.

The conditionally semiessential amino acid taurine possesses a number of cytoprotective properties through...
its actions as an antioxidant, osmoregulator, and intracellular Ca\(^{2+}\) flux regulator. Taurine has been shown to reduce the cell damage associated with the ischemia-reperfusion phenomena (13, 34), to provide the protection against a variety of lung injuries mediated by different toxins (10, 20), and to augment antimicrobial function in normal human neutrophils and monocytes (45). We have previously reported that application of taurine could prevent apoptosis in "tired" human neutrophils (38) and in rat hepatocytes (37). Taurine has been found to be reduced in the plasma in subjects with insulin-dependent diabetes mellitus (17). Moreover, our recent observation that a pharmacological dose of taurine could prevent apoptosis in "tired" human neutrophils (45). We have previously reported that application of taurine attenuates human endothelial cell apoptosis induced by either sodium arsenite or Ca\(^{2+}\) ionophore and prevents endothelial dysfunction (42) has led us to test the hypothesis that taurine may exert a beneficial effect in preventing high-glucose-mediated endothelial cell apoptosis through its antioxidant property and regulation of intracellular Ca\(^{2+}\) homeostasis.

**METHODS**

Reagents

The following reagents were used for the culture of human umbilical vein endothelial cells (HUVEC) and for assessment of HUVEC apoptosis, cytotoxicity, and cell cycle. DMEM, medium 199, Hanks’ balanced salt solution (HBSS) without Ca\(^{2+}\) and Mg\(^{2+}\), FCS, penicillin, streptomycin sulfate, fungizone, glutamine, and 0.05% trypsin-0.02% EDTA solution were purchased from GIBCO BRL (Paisley, Scotland, UK). Sodium acetate, SDS, DMSO, N-acetyl cysteine (NAC), glutathione (GSH), sodium citrate, d-glucose, endothelial cell growth supplement, agarose, 2% gelatin, glyceral, boric acid, bromophenol blue, mannirol, ethidium bromide, N-lauroylsarcosine, EDTA, Tris, HEPES, Triton X-100, propidium iodide (PI), taurine, heparin, paraformaldehyde, and proteinase K were purchased from Sigma (St. Louis, MO). Collagenase (type I) was obtained from Worthington (Freehold, NJ). Bromodeoxyuridine (BrdU), RNase A, and d\(\beta\)X74 DNA/Hae III markers were purchased from Boehringer Mannheim (Mannheim, Germany) and Promega (Madison, WI), respectively.

Cell Culture

HUVEC were isolated by collagenase treatment of umbilical vein and cultured on 2% gelatin-coated culture flasks (Falcon, Lincoln Park, NJ) in complete medium 199 supplemented with 20% FCS, penicillin (100 U/ml), streptomycin sulfate (100 µg/ml), fungizone (0.25 µg/ml), heparin (16 U/ml), endothelial cell growth supplement (75 µg/ml), and 2 mM glutamine as previously described (22). Cells were grown at 37°C in a humidified 5% CO\(_2\) condition and subcultured by trypsinization with 0.05% trypsin-0.02% EDTA when confluency of 80% was reached. Endothelial cells were identified by typical phase-contrast "cobblestone" morphology and by the presence of von Willebrand factor antigen using the immunofluorescence technique. In all experiments reported herein, HUVEC were used as individual isolates between passages 3 and 5.

HUVEC Apoptosis Assay

HUVEC were cultured until 90% confluence was reached. Cells were then treated with high glucose (30 mM) either in the presence or absence of taurine (final concentration 0.125–2.5 mg/ml) and antioxidant DMSO, NAC, GSH (final concentration 0.125–1.0%, 2.5–12.5 mM, and 2.5–12.5 mM, respectively), whereas companion cells (from the same identical individual isolate) were incubated in normal glucose (5.5 mM). To control for medium hyperosmolality, parallel cultures were exposed to 30 mM mannitol. Determination for HUVEC apoptosis by flow cytometry, ELISA kit, and DNA gel electrophoresis were performed following 24 and 48 h of incubation at 37°C in 5% CO\(_2\).

For long-term (14 days) experiments, HUVEC were plated at 1 × 10\(^4\) cells per well in 2% gelatin-coated 12-well, flat-bottom plates (Falcon) and subsequently treated with normal (5.5 mM) and high (30 mM) glucose in the presence or absence of taurine (0.5 mg/ml).

Flow cytometry. HUVEC apoptosis was assessed according to the percentage of cells with hypodiploid DNA by using the PI staining technique as previously described (41, 42). Briefly, after centrifugation, HUVEC (1 × 10\(^4\) cells) in 17 × 100 mm polypropylene tubes (Falcon) were gently resuspended in 1 ml of hypotonic fluorochrome solution (50 µg/ml PI, 3.4 mM sodium citrate, 1 mM Tris, 0.1 mM EDTA, 0.1% Triton X-100) and incubated in the dark at 4°C overnight before they were analyzed by a FACSscan flow cytometer (Becton Dickinson, Mountain View, CA). The forward scatter and side scatter of HUVEC particles were simultaneously measured. The PI fluorescence of individual nuclei with an acquisition of FL2 was plotted against forward scatter, and the data were registered on a logarithmic scale. The minimum number of 5,000 events was collected and analyzed on the software Lysis II. Apoptotic HUVEC nuclei were distinguished by their hypodiploid DNA content from the diploid DNA content of normal HUVEC nuclei. Cell debris were excluded from analysis by raising the forward threshold. All measurements were performed under the same instrument settings.

ELISA kit. An ELISA kit (Boehringer Mannheim), which quantitatively detects cytosolic histone-associated DNA fragments, was employed to assess apoptosis in adhered HUVEC. HUVEC (1.5 × 10\(^4\)) were added in 2% gelatin-coated 96-well, flat-bottom plates (Falcon) and incubated with different treatments. HUVEC DNA fragments were then measured according to the procedures as described in the ELISA kit. Briefly, the cytosolic fraction (13,000 g supernatant) of HUVEC was used as antigen source in a sandwich ELISA with a primary anti-histone monoclonal antibody coated to the microtiter plate and a second anti-DNA monoclonal antibody coupled to peroxidase. The percentage of DNA fragmentation was calculated according to the following formula

\[
\% \text{DNA fragments} = \left(\frac{\text{OD}_{\text{stimulated cell}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control cell}} - \text{OD}_{\text{blank}}}\right) \times 100
\]

where OD is optical density.

DNA gel electrophoresis. Gel electrophoresis for HUVEC DNA fragmentation was carried out according to a modified procedure for assaysing DNA fragmentation in total genomic DNA (41, 42). Briefly, 2 × 10\(^4\) of HUVEC were washed twice with HBSS and pelleted by centrifugation at 250 g for 5 min. The cell pellets were resuspended with 20 µl of lysis buffer (pH 8.0, 20 mM EDTA, 100 mM Tris, and 0.8% N-lauroylsarcosine) and 10 µl of RNase A (pH 4.8, 0.5 mg/ml containing 100 mM sodium acetate and 0.3 mM EDTA) for 6 h at 37°C in a water bath. After treatment with 10 µl proteinase K (20 mg/ml) overnight at 50°C in a water bath, the DNA preparations were added with 5 µl loading buffer (10 mM EDTA, 0.25% bromophenol blue, and 50% glycerol) and were electrophoresed on 1.5% agarose gel containing 0.3 µg/ml ethidium bromide.
bromide in TBE buffer (pH 8.0, 2 mM EDTA, 89 mM Tris, and 89 mM boric acid) for 3 h. HaeIII digest of 6X174 DNA was applied to each gel to provide size markers of 1.4, 1.1, 0.9, 0.6, and 0.3 kilobase pairs, respectively. Gels were photographed under ultraviolet transillumination.

Concomitant Analysis of HUVEC Apoptosis and Cell Cycle

HUVEC apoptosis and cell cycle were simultaneously analyzed using flow cytometric techniques that permit concomitant detection of cell apoptosis and BrdU incorporation at a single-cell level (21). Briefly, HUVEC treated with 30 mM glucose for 48 h were harvested and fixed by a two-step fixation procedure with 2% paraformaldehyde and 70% ethanol. Apoptosis in HUVEC was detected with the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) technique using the in situ cell death detection kit, fluorescein (Boehringer Mannheim). The test is based on the principle that terminal deoxynucleotidyl transferase catalyzes a template-independent addition of deoxynucleotides to free 3' hydroxyl ends present in DNA breaks. The relative number of cells incorporating BrdU, indicating these cells going through the S phase, was measured by supplement of HUVEC with 10 µM BrdU. Cells were then incubated with anti-BrdU mouse monoclonal antibody (DAKO, Glostrup, Denmark) for 30 min and subsequently were stained with R-phycocerythrin-conjugated goat anti-mouse monoclonal antibody (DAKO) for 30 min. HUVEC apoptosis and cell cycle were assessed by a FACSscan flow cytometer (Becton Dickinson) with an acquisition of FL1 for green fluorescence (fluorescein isothiocyanate), indicating DNA fragmentation, and FL2 for orange fluorescence (R-phycocerythrin), indicating BrdU incorporation. The minimum number of 5,000 events was collected and analyzed on the software Lysis II.

HUVEC Cytotoxicity Assay

Lactate dehydrogenase release. HUVEC, seeded in 2% gelatin-coated 24-well, flat-bottom plates (Falcon; 1 × 10^5 cells/well), were incubated with different concentrations of glucose (5.5, 30, 45, and 60 mM) and mannitol (as osmotic control) with or without 0.5 mg/ml taunine at 37°C in 5% CO2 for 12, 24, and 48 h. Lactate dehydrogenase (LDH) activity in the supernatant of HUVEC cultures was measured according to the procedures of LDH Diagnostics Kits (Sigma). Detection of LDH activity was performed on an ultraviolet/visible scanning spectrophotometer (CPU 8720, Philips) at 450 nm. The results of LDH activity were expressed as Berger-Broida units per milliliter.

Statistical Analysis

All data are presented as means ± SD. Statistical analysis was performed using ANOVA. Differences were judged statistically significant when P < 0.05.

RESULTS

Taurine Prevents High-Glucose-Mediated HUVEC Apoptosis

After incubation of HUVEC with 30 mM of either glucose or mannitol for 24 h, no change vs. the effect of 5.5 mM glucose was seen in cell apoptosis (data not shown). However, exposure of HUVEC to high glucose (30 mM) for 48 h resulted in a significant increase in cell apoptosis (P < 0.05, vs. 5.5 mM glucose-treated group), whereas 30 mM mannitol (as osmotic control) failed to induce HUVEC apoptosis (Fig. 1, A and B). Taurine at a pharmacological dose of 0.5 mg/ml prevented high-glucose-mediated HUVEC apoptosis, which was significantly reduced to 7.5 ± 1.4% (P < 0.05, vs. high glucose alone).

An ELISA kit that quantitatively detects DNA fragments was used to determine apoptosis in adhered HUVEC treated with high glucose. As shown in Fig. 1C,
High glucose (30 mM) caused increased DNA fragmentation when compared with 5.5 mM glucose (P < 0.05), whereas taurine (0.5 mg/ml) significantly attenuated this effect (P < 0.05, vs. high glucose alone). In contrast, there was no increase in DNA fragments found in the 30 mM mannitol-treated group.

These findings were further confirmed by DNA gel electrophoresis with the typical “ladder” pattern of DNA fragmentation in those cells treated with 30 mM glucose for 48 h. Taurine abrogated high-glucose-induced DNA fragmentation (Fig. 2). However, antioxidant DMSO, NAC, and GSH only partly attenuated cell apoptosis and DNA fragmentation in HUVEC treated with high glucose (30 mM) for 48 h (Fig. 3, A and B).

Figure 4 shows dose responses of taurine and antioxidant DMSO, NAC, and GSH in attenuation of high-glucose-induced HUVEC apoptosis. Taurine at a dose of 0.5 mg/ml induced maximal and almost complete abrogation of cell apoptosis, whereas DMSO at 0.5%, NAC at 7.5 mM, and GSH at 7.5 mM resulted in maximal but partial inhibition of cell apoptosis.

Exposure of HUVEC to high ambient glucose (30 mM) for 48 h (short-term incubation), as well as for 14 days (long-term incubation), significantly increased DNA fragments compared with normal glucose at 5.5 mM (P < 0.05) (Fig. 5). However, increasing the time span of incubation with 30 mM glucose from 48 h to 14 days did not significantly augment the degree of DNA fragmentation (624 ± 168% DNA fragments of control in long-term experiments vs. 509 ± 174% DNA fragments of control in short-term experiments). Taurine consistently prevented high-glucose-induced DNA fragmentation after long-term incubation (Fig. 5).

By concomitant analysis of HUVEC apoptosis and BrdU incorporation with flow cytometry, Fig. 6 clearly shows that apoptotic cells with DNA strand breaks, induced by exposure to high glucose (30 mM) for 48 h, preferentially occurred in the S phase, as the FL1-
fluorescence-positive HUVEC (indicating DNA fragmentation) were simultaneously positive for BrdU staining (FL2 fluorescence positive).

Taurine Attenuates High-Glucose-Mediated Increased Intracellular ROS and \([\text{Ca}^{2+}]_i\)

To elucidate the possible mechanisms by which taurine prevented high-glucose-mediated HUVEC apoptosis, we measured intracellular ROS and \([\text{Ca}^{2+}]_i\) after HUVEC were incubated with high glucose in the presence or absence of taurine. Exposure of HUVEC to high glucose at 30 mM for 24 h significantly enhanced intracellular ROS formation from 51 ± 6 MCF (mean channel fluorescence per cell) in the 5.5 mM glucose group to 102 ± 25 MCF (Fig. 7) and elevated the \([\text{Ca}^{2+}]_i\) level from 94 ± 15 MCF under control conditions to 131 ± 13 MCF (P < 0.05, vs. 5.5 mM glucose; Fig. 8). However, 30 mM mannitol had no effect on both intracellular ROS and \([\text{Ca}^{2+}]_i\). The addition of taurine (0.5 mg/ml) attenuated high-glucose-mediated increased intracellular ROS formation as well as the elevated \([\text{Ca}^{2+}]_i\) level (Figs. 7 and 8).

Taurine Does Not Prevent Hyperosmolarity-Induced HUVEC Necrosis

Glucose at 30 mM, which induced apoptosis, did not cause HUVEC injury as represented by LDH release following 24 and 48 h incubation (Fig. 9, A and C). However, glucose at 60 mM resulted in a significant increase in HUVEC LDH release. Similarly, mannitol at 60 mM also induced LDH release (Fig. 9, B and D). As shown in Table 1, both glucose and mannitol at 60 mM induced small but significant HUVEC lysis after 48 h of incubation. Taurine failed to prevent hyperosmolarity-mediated cell necrosis (Fig. 9 and Table 1).

DISCUSSION

The studies performed here have documented that exposure of cultured HUVEC to high ambient glucose (30 mM, which was chosen to match glucose levels prevalent in uncontrolled diabetic patients) for either 48 h (short term) or 14 days (long term) resulted in an increased apoptosis, as confirmed by flow cytometry analysis, DNA fragment-detection ELISA, and DNA gel electrophoresis. More importantly, the application of a pharmacological dose of taurine (0.5 mg/ml) significantly prevented high-glucose-induced endothelial cell apoptosis both after short-term and long-term incubation, which was correlated with taurine attenuation of high-glucose-mediated increased intracellular ROS formation and elevated \([\text{Ca}^{2+}]_i\) levels.

High-glucose-triggered human endothelial cell apoptosis appears to be selective and specific, because no increase in apoptosis was seen in HUVEC treated with...
the osmotic control mannitol. Furthermore, Baumgartner-Parzer et al. (7) have found that high glucose at 30 mM, which is responsible for inducing human endothelial cell apoptosis, fails to cause apoptosis in different standardized cell lines (K-562, P815, YT) and fibroblasts. On the other hand, a potential increase of apoptosis in HUVEC exposed to 30 mM glucose was found not only following 48-h incubation, but also following long-term (14 days) incubation, which is consistent with another report of HUVEC apoptosis at 20% after 48 h and at 46% after long-term (13 ± 1 days) high-glucose exposure (7). These results indicate that cultured HUVEC may not be able to acquire the cellular adaptation for minimizing the detrimental effect of high ambient glucose on HUVEC following long-term exposure.

![Fig. 4](image-url)  
**Fig. 4.** Dose responses of taurine and antioxidants in attenuation of high-glucose-induced HUVEC apoptosis. HUVEC were incubated with high glucose (HG, 30 mM) in the presence or absence of either taurine (Tau) or antioxidant DMSO, NAC, or GSH at 37°C in 5% CO2 for 48 h. HUVEC apoptosis was assessed according to percent of cells with hypodiploid DNA by flow cytometry as described in METHODS. Data are representative of 3 separate experiments.

![Fig. 5](image-url)  
**Fig. 5.** Induction of HUVEC apoptosis after long-term (14 days) exposure to high glucose. HUVEC were treated with normal glucose (NG, 5.5 mM), high mannitol (HM, 30 mM), high glucose (HG, 30 mM), and high glucose + taurine (Tau, 0.5 mg/ml) at 37°C in 5% CO2 for 14 days. DNA fragmentation in HUVEC was assessed by ELISA kit as described in METHODS. Data are expressed as means ± SD and are representative of 6 separate experiments. Statistical significance was compared with NG (*P < 0.05) and with HG alone (@P < 0.05).

![Fig. 6](image-url)  
**Fig. 6.** Concomitant detection of HUVEC DNA fragmentation and measurement of bromodeoxyuridine (BrdU) incorporation. HUVEC were incubated with 30 mM glucose at 37°C in 5% CO2 for 48 h. DNA strand breaks in HUVEC and the relative BrdU content were simultaneously detected by in situ TUNEL kit and anti-BrdU monoclonal antibody as described in METHODS. FL1 fluorescence positive on x-axis represented relative cell population with DNA fragmentation, whereas FL2 fluorescence positive on y-axis represented relative number of cells incorporating BrdU and going through the S phase. Top right quartet: high-glucose-triggered HUVEC apoptosis preferentially occurred in the S phase cells.

![Fig. 7](image-url)  
**Fig. 7.** Taurine attenuates high-glucose-induced HUVEC intracellular reactive oxygen species (ROS) generation. HUVEC were treated with normal glucose (NG), high glucose (HG), and high mannitol (HM) in the presence or absence of taurine (Tau, 0.5 mg/ml) at 37°C in 5% CO2 for 24 h. ROS formation in HUVEC was detected as described in METHODS and expressed as mean channel fluorescence per cell (MCF). Data are presented as means ± SD and are representative of 6 separate experiments. Statistical significance was compared with NG (*P < 0.05) and with HG alone (@P < 0.05).
Recent observations have revealed a profound regulatory interrelationship between programmed cell death and the cell cycle. Boehme et al. (8) reported that mature T lymphocytes blocked in the S phase with aphidicolin or excess thymidine were susceptible to T cell receptor-induced apoptosis. Topoisomerase inhibitor-mediated DNA strand breaks in HL-60 cells were detected in the cells that were going through the S phase (19). In this study we observed that high-glucose-triggered HUVEC apoptosis appeared preferentially to occur in the S phase, as confirmed by the technique of concomitant analysis for HUVEC DNA strand breaks and the cell cycle on flow cytometry.

Human endothelial cell necrosis, another form of cell death, may also contribute to high-glucose-triggered accelerated endothelial cell death. However, as demonstrated in the present study, glucose at 30 mM, which induced apoptosis, did not cause HUVEC necrosis as represented by LDH release and cell lysis. Both glucose and mannitol at 60 mM resulted in a hyperosmolality-associated HUVEC necrosis, whereas taurine failed to prevent this hyperosmolality-induced cell necrosis.

The present study suggests that high glucose levels may have a detrimental effect on endothelial cells. This conclusion is supported by the observations of Baumgartner-Parzer et al. (7), who also demonstrated that in vitro elevated glucose levels trigger human

![Fig. 8](image1.png)

**Fig. 8.** Taurine prevents high-glucose-mediated HUVEC intracellular ionized Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) elevation. HUVEC were treated with normal glucose (NG), high glucose (HG), and high mannitol (HM) in the presence or absence of taurine (Tau, 0.5 mg/ml) at 37°C in 5% CO\(_2\) for 24 h. HUVEC [Ca\(^{2+}\)]\(_i\) level was assessed as described in METHODS and expressed as MCF. Data are presented as means ± SD and are from 6 separate experiments. Statistical significance was compared with NG (*P < 0.05) and with HG alone (@P < 0.05).

![Fig. 9](image2.png)

**Fig. 9.** Effect of high glucose and mannitol on HUVEC lactate dehydrogenase (LDH) release. HUVEC were treated with different concentrations of glucose (A and C) and mannitol (B and D) with or without taurine (Tau, 0.5 mg/ml) for 24 and 48 h. LDH release in supernatants of HUVEC cultures was measured as described in METHODS. ○, glucose; ●, glucose + Tau; ▲, mannitol; ▼, mannitol + Tau, respectively. Results are expressed as means ± SD and are representative of 4 separate experiments. Each experiment was carried out in triplicate. Statistically significant differences were compared with NG (5.5 mM). *P < 0.05 vs. LDH level in normal glucose group (183 ± 43 U/ml) after 24 h incubation; @P < 0.05 vs. LDH level in normal glucose group (222 ± 46 U/ml) after 48 h incubation.

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<th>Glucose and mannitol-induced HUVEC lysis</th>
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<td><strong>Glucose</strong></td>
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<td>30 mM</td>
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<td>−Tau 0.3 ± 2.3</td>
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Values (in %) are means ± SD and are representative of 4 separate experiments. Each experiment was carried out in triplicate. Human umbilical vein endothelial cells (HUVEC; 2 × 10\(^4\)) labeled with \(^{51}\)Cr were seeded per well of gelatin-coated 96-well, flat-bottom plates and were incubated with glucose and mannitol in the presence or absence of taurine (Tau, 0.5 mg/ml) at 37°C in 5% CO\(_2\) for 48 h. HUVEC lysis was assessed as \(^{51}\)Cr release as described in METHODS. Statistically significant difference was compared with normal glucose (5.5 mM, cell lysis = 0 ± 0), *P < 0.05.
endothelial cell apoptosis, and by the in vivo findings (35) that accelerated retinal microvascular cell apoptosis occurs in human and experimental diabetic retinopathy. However, the underlying mechanisms by which high glucose induces programmed cell death in human vascular endothelium need to be elucidated. Two mechanisms could be proposed, based on the findings in the present study, that may explain how hyperglycemia triggers endothelial cell apoptosis. One mechanism involves the oxidative stress response that has been recently recognized as a mediator in the induction of cell apoptosis. Sodium arsenite, a potent activator for the transcription of heme oxygenase, which is a major representative of the oxidative stress response (4), induced a dose-dependent increase in endothelial cell apoptosis (41). Scavenging of intracellular ROS, such as the hydroxyl radical, or augmenting intracellular reduced GSH levels with membrane-permeable antioxidants significantly blocks apoptosis in endothelial cells (1, 2). Hyperglycemia in diabetes may cause increased ROS production in endothelial cells through autoxidation of glucose, formation of intermediate products of cyclooxygenase catalysis, or mitochondrial respiration (12). Moreover, an impaired radical scavenger function via the GSH reduct cycle in endothelial cells exposed to high glucose has been reported (5, 23), which may also contribute to excessive intracellular ROS accumulation. As clearly evidenced in this study, high glucose at 30 mM significantly enhanced intracellular ROS formation in HUVEC after 24-h incubation, which correlated with subsequent cell apoptosis. Furthermore, blockage of intracellular ROS with antioxidants DMSO, NAC, and GSH could partly prevent high glucose-mediated HUVEC apoptosis, indicating involvement of increased intracellular ROS in hyperglycemia-triggered DNA fragmentation in HUVEC.

$[Ca^{2+}]_i$ is believed to play a crucial role in mediating cell apoptosis, which may possesses another mechanism for hyperglycemia-mediated programmed cell death in vascular endothelium. Preventing or modulating the increase in $[Ca^{2+}]_i$, by utilizing either $Ca^{2+}$-free media or intra- or extracellular $Ca^{2+}$ chelators [EGTA and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid], ameliorates programmed cell death (40). On the other hand, many inducers of cell apoptosis clearly increase $[Ca^{2+}]_i$, and the addition of $Ca^{2+}$-ionophores (A-23187 and ionomycin) readily induce cell apoptosis (3). In the present study, we have demonstrated that incubation of HUVEC with elevated glucose results in a direct increase in $[Ca^{2+}]_i$ levels. Similarly, Wascher et al. (44) also reported that pathologically high glucose enhanced $Ca^{2+}$ mobilization either through increased release of intracellular stored $Ca^{2+}$ or through increased $Ca^{2+}$ influx in endothelial cells.

The most important finding in this study is that taurine at its pharmacological dose significantly prevents pathologically high-glucose-induced HUVEC apoptosis both after short-term and long-term incubation. Because taurine simultaneously attenuates high-glucose-mediated increased intracellular ROS generation as well as elevated $[Ca^{2+}]_i$ levels, we propose that the mechanisms by which taurine attenuates high-glucose-triggered HUVEC apoptosis may be in part, if not totally, through its antioxidant activity and $[Ca^{2+}]_i$ modulation. First, taurine can act as a direct antioxidant that scavenges or quenches oxygen free radicals intracellularly to block ROS-mediated programmed cell death. Our previous work has shown that taurine abrogation of sodium arsenite-induced human endothelial cell apoptosis is associated with its antioxidant property (42). Other investigators (13, 34) have shown the beneficial effects of the ROS-scavenging capacity of taurine, specifically in relation to attenuation of lipid peroxidation, reduction of membrane permeability, and inhibition of intracellular oxidation in different cells. Apart from its effect on antioxidant defense, taurine also functions principally as a modulator of intracellular $Ca^{2+}$ homeostasis. Taurine appears to affect cell metabolism through a $Ca^{2+}$ biphasic effect that depends on $Ca^{2+}$ concentration (39). Taurine has been found to prevent increased $[Ca^{2+}]_i$-mediated cell injury (13, 36, 39). Furthermore, we have previously demonstrated (42) that taurine protects against $Ca^{2+}$-ionophore (A-23187)-induced human endothelial cell apoptosis.

In summary, although its precise mechanism of action has not yet been thoroughly elucidated, the data from the present study demonstrate that taurine is capable of preventing pathologically high-glucose-triggered accelerated programmed cell death in human vascular endothelial cells, possibly through intracellular ROS inhibition and $[Ca^{2+}]_i$ stabilization. The findings that high glucose specifically induces an accelerated apoptosis in HUVEC following a short term of incubation, as evidenced by the present study and another study (7), and that endothelial cells and pericytes are dying by apoptosis in the very early stage of diabetic retinopathy, which may eventually lead to retinal capillary obliteration (35), indicate that hyperglycemia-triggered accelerated vascular endothelial cell apoptosis is an early and crucial event in the pathogenesis of diabetes-associated microangiopathy. Therefore, the finding that the beneficial effect of taurine on the prevention of pathologically high-glucose-induced human vascular endothelial cell apoptosis suggests that this amino acid may have important implications for the pharmacological manipulation of diabetes-associated microvascular complications.

Address for reprint requests and other correspondence: H. P. Redmond, Dept. of Surgery, Cork Univ. Hospital, Univ. College Cork, Cork, Ireland (E-mail: p.redmond@ucc.ie).

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