DIABETES/METABOLISM RESEARCH AND REVIEWS
Diabetes Metab Res Rev 2008; **24**: 301–309.
Published online 19 December 2007 in Wiley InterScience (www.interscience.wiley.com) **DOI:** 10.1002/dmrr.804

RESEARCH ARTICLE

Evaluation of gliclazide ability to attenuate the hyperglycaemic 'memory' induced by high glucose in isolated human endothelial cells

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Received: 16 April 2007
Revised: 3 August 2007
Accepted: 29 September 2007

Abstract

Background Patients with long-term exposure to high levels of hyperglycaemia remain more susceptible to diabetes-related complications, even with subsequent lower levels of hyperglycaemia. We sought to confirm the hypothesis that exposure to continuous increased glucose results in a memory of cellular stress in isolated endothelial cells, even when switched back to normal glucose, and to investigate the ability of gliclazide to attenuate this phenomenon.

Methods Human umbilical vein endothelial cells were incubated for 21 days in normal glucose (5 mmol/L), high glucose (30 mmol/L), or high glucose for 14 days followed by normal glucose for 7 days (memory condition). The effects of gliclazide (10 μmol/L) and glibenclamide (1 μmol/L) were evaluated in the memory condition and added to the culture media early (first 14 days), late (last 7 days), or throughout the study. Oxidative stress and cell apoptosis parameters were investigated.

Results Continuous high glucose increased reactive oxygen species, 8-OHdG, nitrotyrosine, caspase-3, and reduced Bcl-2 expression. These deleterious effects were also observed in the memory condition. Gliclazide applied early or throughout the study improved all parameters. In contrast, glibenclamide showed no relevant effect on study parameters.

Conclusions Our results suggest that gliclazide prevents endothelial cell apoptosis by reducing oxidative stress. The results appear to confirm the hypothesis that exposure of cells to continuous increased glucose results in a hyperglycaemic cellular memory that remains, even when cells are switched back to normal glucose. Gliclazide attenuated this cellular memory, decreasing oxidative stress and protecting vascular endothelial cells from apoptosis. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords hyperglycaemic memory; endothelial cells; oxidative stress

Introduction

Hyperglycaemia is the hallmark metabolic abnormality of diabetes and its involvement in the pathogenesis of diabetes complications is undisputed. Oxidative stress is thought to be the underlying cause of both the macrovascular and microvascular hyperglycaemia-induced complications associated with diabetes mellitus [1].

A number of years ago the possibility that a 'hyperglycaemic memory' for a hyperproduction of fibronectin and collagen in endothelial cells persisting after glucose normalization, was preliminarily reported [2]. Using the same design, by a culture of endothelial cells for 14 days in high glucose followed by 7 days in normal glucose, we have recently shown that an overproduction of free radicals persists after normalization of glucose. This is accompanied by a prolongation of the induction of protein kinase C (PKC)-β, NAD(P)H oxidase, Bax, collagen and fibronectin, and 3-nitrotyrosine [3], suggesting that oxidative stress may be involved in the 'metabolic memory' effect. Further evidence for this hypothesis is provided by the observation that this metabolic memory can be reversed using a variety of antioxidant molecules [3].

Gliclazide, a second-generation sulfonylurea, reduces blood glucose levels by augmenting insulin release from pancreatic islet cells. Besides its hypoglycaemic effect, gliclazide has been shown to possess antioxidant properties [4,5]. It appears that these antioxidant effects of gliclazide are independent of any effects on glycaemic control [6].

The objectives of the current study were twofold. First, to confirm that exposure to continuous increased glucose results in a memory of cellular stress in isolated endothelial cells, even when they are switched back to normal glucose. Second, to evaluate the ability of gliclazide to attenuate this hyperglycaemic memory in human endothelial cells.

Research design and methods

Isolation and culture of human endothelial cells

Human umbilical vein endothelial cells (HUVECs) were isolated and pooled from umbilical cords obtained from normal vaginal deliveries by the procedure described by Jaffe *et al*. [7]. Cells were cultured in gelatine-coated 60 mm Petri dishes (Sarstedt, Newton, NC, USA) and grown in medium 199 (GIBCO BRL, Gaithersburg, MD, USA) supplemented with 2 mmol/L glutamine (GIBCO), 20% heat inactivated fetal bovine serum (GIBCO), 25 μg/mL endothelial cell growth supplement, 90 μg/mL heparin (GIBCO), and 0.25 μg/mL Fungizone (GIBCO). Petri dishes were incubated at 37 °C in 5% CO_2 per 95% air gas mixture.

Primary cultures were fluid changed 24 h after seeding and were sub-cultured on reaching confluence with 0.01% trypsin ethylenediaminetetraacetic acid (EDTA), inactivated by dilution. Cultured cells were identified as endothelial by their morphology and the presence of factor VIII-related antigen detected using indirect immunofluorescence as previously described by Risso *et al*. [8].

Only first and second passage HUVECs were used in the study to avoid age-dependent cellular modifications. HUVECs were seeded at equal density (1.3×10^5) in gelatine-coated 60 mm Petri dishes and allowed to attach overnight. Then they were exposed to one of three experimental conditions [3]:

(1) Continuous normal glucose (5 mmol/L) for 21 days
(2) Continuous high glucose (30 mmol/L) for 21 days
(3) Continuous high glucose (30 mmol/L) for 14 days followed by normal glucose (5 mmol/L) for 7 days.

Gliclazide and glibenclamide at therapeutic concentrations, 10 μmol/L and 1 μmol/L, respectively were added to the third condition, early (first 14 days), late (last 7 days) or throughout the study. Osmotic control was ensured by incubating cells with mannitol at the same concentration as glucose. The superoxide scavenger Mn (III) tetrakis (4-benzoic acid) porphyrin chloride (MnTBAP) 100 μmol/L (Calbiochem, Darmstadt, Germany) was used for comparative antioxidant control.

Western blot analyses

Whole cells were lysed using M-PER lysis buffer (Pierce Chemicals) for endothelial cells with 150 mmol/L NaCl and containing protease inhibitor cocktail (Pierce Chemicals) and phosphatase inhibitor cocktail II (Sigma). Protein expression was evaluated by Western blot analysis using specific antibodies (details in the specific paragraphs). Equal amounts of protein lysates (determined by Pierce micro BCA protein assay 30 or 50 μg) were fractionated by SDS-PAGE (5–12% gradient gels), and proteins were transferred to a 0.45 μm PVDF transfer membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membrane was blocked with 1% low fat milk in Tris-buffered saline (TBS) containing 0.05% Tween-20 or SuperBlock (Pierce Chemicals) and subsequently incubated with the primary antibody at a suitable dilution overnight at 4 °C or for 2 h at room temperature. Antibodies for Bcl-2, Caspase-3 and Actin were from Santa Cruz Biotechnology, for Catalase from Calbiochem and for Fibronectin from Sigma.

Unbound antibody was removed by washing three times in TBS containing 0.05% Tween-20. SuperSignal Dura chemiluminescence substrate (Pierce Chemicals) was added for detection. Membranes were subsequently exposed to Kodak Bio Max Light Film and the intensity of Western blot signals was quantified by densitometry using Image J 1.30 analysis software, calibrating the image using uncalibrated optical density values and then integrating intensity and area. In all cases, confirmation of equal loading was accomplished by staining blots with Ponceau S and by probing blots against the cytoskeleton protein actin.

Cell viability

Cell viability was assessed with Cell Titer Blue Viability Assay (Promega). This assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Non-viable cells

DOI: 10.1002/dmrr

rapidly lose metabolic capacity and thus do not generate a fluorescent signal. The homogeneous assay procedure involves adding the single reagent directly to cells cultured in serum-supplemented medium. After an incubation step (2–4 h), data are recorded using a spectrofluorometer (Tecan).

Markers of apoptosis

Bcl-2 expression

Levels of Bcl-2, an anti-apoptotic protein, in HUVEC cell lysates were detected by the use of a commercial ELISA kit (Bender MedSystems Diagnostics, Vienna, Austria), according to the manufacturer's instructions. The quantification was performed with a Western blot assay, carried out using a specific mouse monoclonal IgG anti-Bcl-2 antibody (Santa Cruz), diluted 1 : 100 as described for PKC-β isoforms.

Caspase-3 expression

The activity of caspase-3, an apoptosis-related cysteine peptidase, was assayed by the use of Chemicon's CPP32/Caspase-3 Colorimetric Protease Assay Kit (Chemicon International, Temecula, CA). The assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide after cleavage from the labelled substrate DEVD-*p*-nitroanilide. Caspase-3 expression was performed with a Western blot assay as described below for PKC-β isoforms using a specific rabbit polyclonal IgG anti-caspase-3 antibody (Santa Cruz), diluted 1 : 200.

Oxidative stress

8-OHdG ELISA

8-OHdG was determined in HUVEC DNA digests using Bioxytech 8-OHdG-EIA Kit, a competitive ELISA (OXIS Health Products, Portland, OR, USA). HUVEC DNA was isolated using DNAzol Reagent (GIBCO BRL, Life Technologies, Grand Island, NY, USA) according to manufacturer's instructions and quantified using a spectrophotometer. Samples containing 400 μg of DNA were resuspended in 50 μL of reaction mixture, containing 100 mmol/L sodium acetate (pH 5.0) and 5 mmol/L $MgCl_2$ and digested with 1 μL of DNAse I (Sigma Aldrich, St. Louis, Missouri, USA) for 10 min at room temperature. DNA-digested samples were added to the microtitre plate pre-coated with 8-OHdG, and the assay was performed according to manufacturer's instructions.

Nitrotyrosine measurement

After 21 days, culture cells were lysed as reported in the Western blot assays, and protein content was determined by the Bio-Rad protein assay kit. An identical amount of protein (50 μg) was applied to a Maxisorp ELISA plate (NUNC Brand Products) together with nitrated bovine serum albumin standard samples (SIGMA) using 50 mmol/L Na_2CO-$NaHCO_3$ buffer at pH 9.6, and allowed to bind overnight at 4 °C. Afterwards, non-specific binding sites were blocked with 1% bovine serum albumin in phosphate buffered saline. The wells were incubated at 37 °C for 1 h with a mouse monoclonal antibody anti-nitrotyrosine (Upstate Biotechnology, Lake Placid, NY) (5 μg/mL) and then for 45 min at 37 °C with a peroxidase-conjugated goat anti-mouse IgG secondary antibody diluted 1 : 1000. After washing the plates, the peroxidase reaction product was generated using a TMB peroxidase substrate. Plates were incubated for 10 min at room temperature and the reaction was stopped with 50 μL per well of H_2SO_4 0.5 mol/L. Signals were recorded with Tecan microplate reader at 450 nm.

Cell-signalling pathways

NAD(P)H oxidase

NAD(P)H oxidase expression was evaluated through the detection of its components p22phox and p47phox by the Western immunoblotting technique as described below for PKC-β isoforms. Specific antibodies against the two NAD(P)H components were purchased from Santa Cruz Biotechnology, and they were used at the following dilutions: goat polyclonal anti-p22phox (1 : 100) and goat polyclonal anti-p47phox (1 : 100). Secondary anti-goat IgG antibody (Santa Cruz) was used at 1 : 1000 dilution.

Protein kinase C activity

Detection of PKC activity was performed with PepTag assay (Promega Corporation, Madison, WI, USA), which uses brightly coloured, fluorescent peptide substrate that is highly specific for PKC. Phosphorylation by PKC of the specific substrate alters the peptide's net charge from +1 to −1. Samples were separated on a 0.8% agarose gel at 100 V for 15 min. The phosphorylated peptide migrated towards the anode (+), and the non-phosphorylated peptide migrated towards the cathode (−). The gel was photographed on a UV transilluminator. Quantification of results was performed by densitometry.

PKC-βI and PKC-βII expression

Assays were performed by Western blot analysis with specific antibodies directed against PKC-βI and PKC-βII isoforms (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Cells were washed three times in cold PBS and then lysed for 30 min at 4 °C in buffer containing 1% Non-idet P-40, 50 mmol/L Tris-HCl (pH 7.5), 100 mmol/L NaCl, 5 mmol/L ethylenediaminetetraacetic acid and 1 mmol/L phenylmethylsulfonyl fluoride. After centrifugation at 13 000 g for 10 min at 4 °C; the supernatant was collected and the protein contents of all the samples were determined by the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). An identical amount of protein (20 μg) for each lysate was subjected to SDS-PAGE. The concentration of polyacrylamide gels was 8%. Proteins were transferred to a nitrocellulose membrane

DOI: 10.1002/dmrr

(Amersham Pharmacia Biotech, Buckinghamshire, UK) using the Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad Laboratories). The filters were blocked for 2 h using 5% non-fat dried milk in TBS (50 mmol/L Tris, 0.15 mol/L NaCl [pH 7.5]) containing 0.1% Tween 20, washed with TBS and incubated at room temperature for 2 h with the primary antibody at a suitable dilution: mouse monoclonal anti-cPKC-βI (1 : 100), rabbit polyclonal anti-cPKC-βII (1 : 200). Filters were later washed with TBS and incubated with 1 : 1000 dilution of secondary anti-mouse or anti-rabbit IgG antibody, coupled with horseradish peroxidase. The enhanced chemiluminescence system (Amersham Pharmacia Biotech) was used for detection. Filters were subsequently exposed to Kodak Bio Max Light-1 films, and the intensity of Western blot was quantified by densitometry.

Statistical methods

All data are mean ± SD. One-way analysis of variance with the Neuman-Keuls post-hoc test was used to determine differences between the mean values of groups. Differences were considered significant at $P < 0.05$.

Results

Cell viability

In all test conditions, mannitol-treated cells (osmotic control) were undistinguishable from normal glucose-treated cells. Cell viability was lower in cells cultured in high glucose for 21 days ($P < 0.001$) and in high glucose for 14 days followed by normal glucose for 7 days ($P < 0.001$; the memory condition) when compared with cells grown in normal glucose for 21 days (Figure 1). The addition of gliclazide to the memory condition improved cell viability ($P < 0.01$); the greatest improvement was seen when gliclazide was added throughout the 21 days. Addition of glibenclamide had no effect on cell viability compared with non-drug treated cells grown in the memory condition. The addition of the MnSOD mimetic, MnTBAP, after 14 days of high glucose normalized cell viability ($P < 0.01$) (Figure 1).

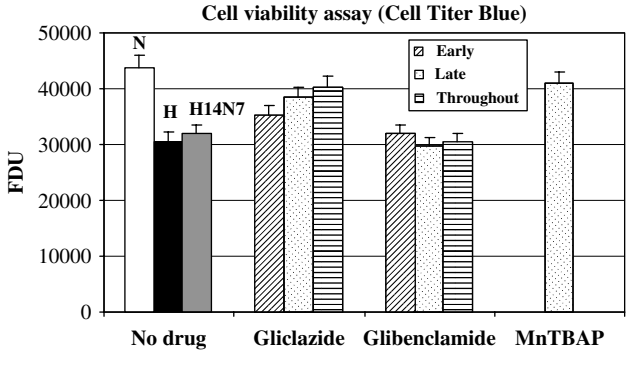

Figure 1. Cell Titer Blue viability assay showing viability of the cells (expressed as fluorescence units) cultured in normal (N) glucose (5 mmol/L) for 21 days; high (H) glucose (30 mmol/L) for 21 days; and high glucose for 14 days followed by normal glucose for 7 days (H14N7). In this 'memory' condition, gliclazide or glibenclamide was added early (E, 1–14 days), late (L, 14–21) or throughout (T) 21 days of exposure. MnTBAP was added only late (last 7 days). Bars indicate standard deviation

Markers of apoptosis

Bcl-2 expression

Western blot analysis showed that Bcl-2 protein expression decreased in cells cultured in high glucose (30 mmol/L) ($P < 0.001$) or the memory condition ($P < 0.001$) compared with the normal glucose condition (5 mmol/L). The addition of gliclazide in cells cultured in the memory condition increased Bcl-2 expression whether the drug was added early (1–14 days), late (14–21 days) or throughout ($P < 0.001$) (Figure 2). The effect was most pronounced when cells were exposed throughout 21 days. Bcl-2 expression following the addition of glibenclamide was similar to levels observed in non-drug treated cells cultured in the memory condition. The addition of MnTBAP normalized levels of Bcl-2 expression ($P < 0.001$) (Figure 2).

Caspase-3 expression

Caspase-3 levels doubled in cells cultured in high glucose (30 mmol/L) for 21 days compared with cells cultured in the normal condition ($P < 0.001$). Caspase-3 expression in cells cultured in the memory condition was intermediate between the normal and high-glucose conditions. Gliclazide significantly decreased caspase-3 expression in all the test conditions (Figure 2). The decrease was most marked when the drug was added early. Glibenclamide had no effect on caspase-3 expression compared with the memory condition, whereas MnTBAP prevented the increase in caspase-3 expression ($P < 0.01$) (Figure 2).

Basement membrane thickening

Fibronectin

Fibronectin expression increased in cells cultured in high glucose or in the memory condition. Gliclazide treatment decreased levels of fibronectin expression ($P < 0.01$), especially when the drug was added late (Figure 3). There was no effect of glibenclamide treatment. MnTBAP normalized levels of fibronectin expression ($P < 0.01$).

Oxidative stress

8-OHdG DNA adducts

Levels of 8-OHdG DNA adducts rose in cells exposed to high glucose for 21 days and even more in cells exposed to the memory condition ($P < 0.001$ for both

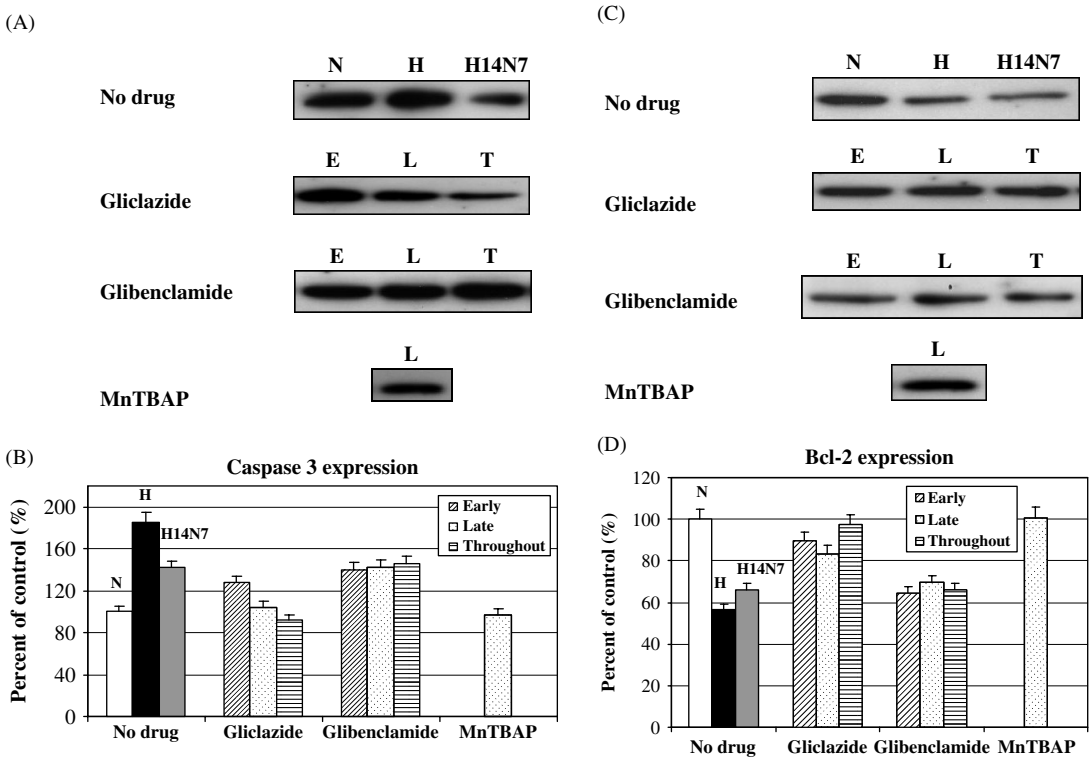

Figure 2. Caspase-3 and Bcl-2 expression. (A–C) Representative Western blot analysis after 21 days of experiment. (B–D) Quantitation of signal intensity performed by densitometry. Bars indicate standard deviation. Cytoskeleton protein actin has been used as reference

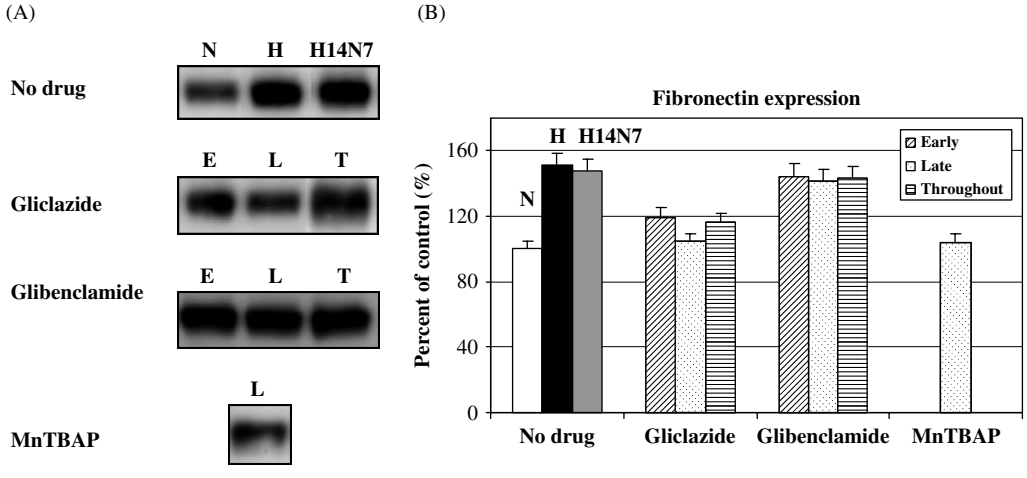

Figure 3. Fibronectin expression. (A) Representative fibronectin Western blot after 21 days of experiment. (B) Quantitation of signal intensity by densitometry. Bars indicate standard deviation. Quantification of actin has been used as a reference

conditions). Levels of 8-OHdG were reduced in gliclazide-treated cells, whether the drug was added early, late or throughout 21 days ($P < 0.001$) (Figure 4). The effect was most pronounced when the drug was added throughout. Glibenclamide administration had no significant protective effect. Levels of 8-OHdG were normalized following addition of MnTBAP ($P < 0.001$) (Figure 4).

Nitrotyrosine measurements

Exposure of cells to high glucose or to the memory condition produced an increase in nitrotyrosine content ($P < 0.001$). Nitrotyrosine content was reduced in cells treated with gliclazide ($P < 0.001$), especially when the drug was added throughout the experimental period (21 days) (Figure 4). Glibenclamide treatment had no effect. In contrast, nitrotyrosine levels in MnTBAP-treated cells were similar to those in cells cultured in normal glucose conditions ($P < 0.001$) (Figure 4).

Expression of antioxidant enzymes

Expression of the antioxidant enzymes MnSOD and catalase was increased in cells cultured in high-glucose and

DOI: 10.1002/dmrr

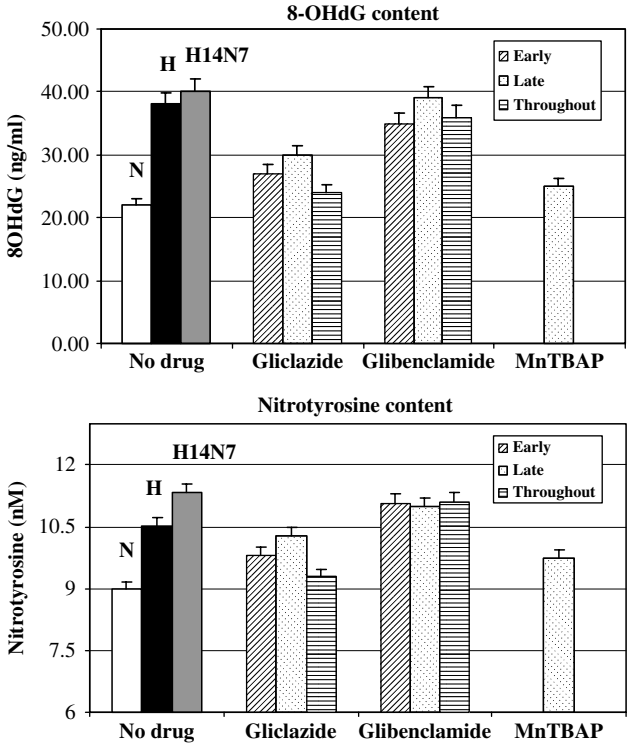

Figure 4. Amount of 8-OHdG and nitrotyrosine in HUVEC after 21 days of experiment (bars show standard deviation)

memory conditions compared with cells cultured in normal glucose ($P < 0.001$). Gliclazide reduced cell expression of MnSOD and catalase ($P < 0.001$), particularly when added throughout the 21-day experimental period, whereas glibenclamide had no relevant effect (Figure 5). Antioxidant enzyme expression was reduced to control levels in cells treated with MnTBAP ($P < 0.001$).

Cell-signalling pathways

Protein kinase C activity and expression

After 21 days, the activity of PKC showed a marked increase in cells exposed to high-glucose and memory conditions ($P < 0.001$). Addition of gliclazide to the media reduced PKC activation ($P < 0.001$), whereas glibenclamide had not effect (Figure 6). MnTBAP produced a similar reduction in PKC activity to gliclazide ($P < 0.001$). The Western blot analysis of electrophoresed proteins confirmed that expression of the two PKC isoforms PKC-βI and PKC-βII was increased in high-glucose and memory conditions ($P < 0.001$). Expression was reduced by gliclazide ($P < 0.001$), particularly when cells were exposed throughout the 21-day period (Figure 7). Glibenclamide had no relevant effect, whereas addition of MnTBAP to the media reduced expression to levels observed in normal glucose controls ($P < 0.001$).

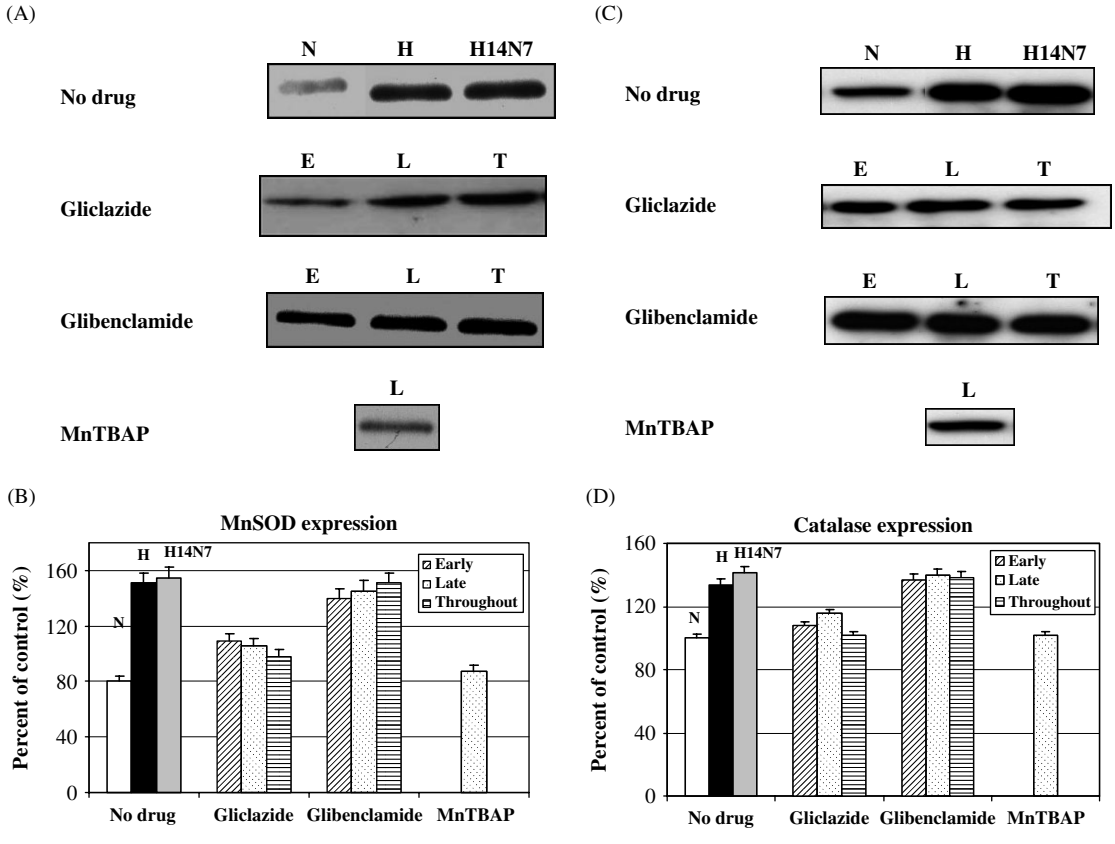

Figure 5. Representative Western blot of MnSOD (A) and Catalase (C) after 21 days of experiment. B and D indicate the quantitation of signal intensity of MnSOD and Catalase, respectively, performed by densitometry. Bars indicate standard deviation. Cytoskeleton protein actin has been used as a reference

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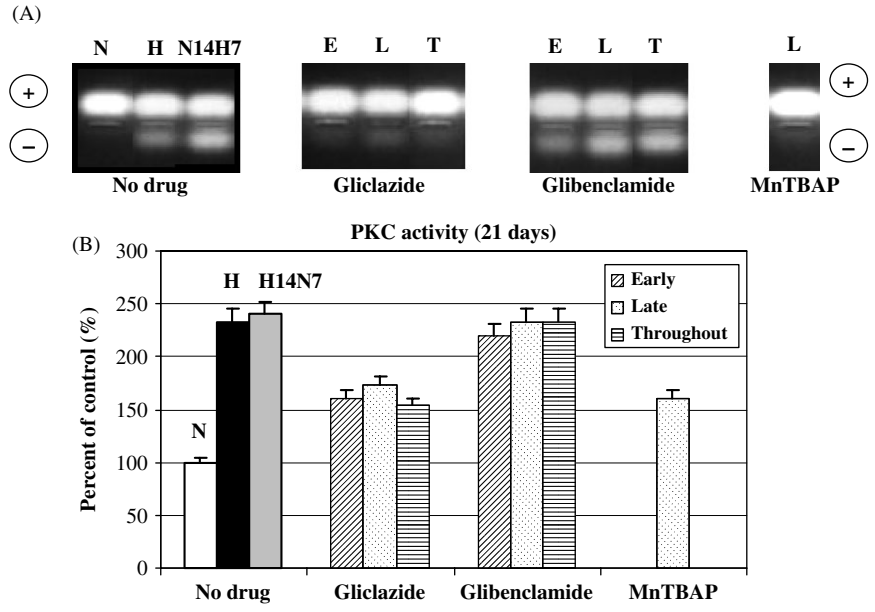

Figure 6. (A) Detection of PKC activity. (B) PKC activity calculated by densitometry; bars indicate standard deviation. Cytoskeleton protein actin has been used as a reference

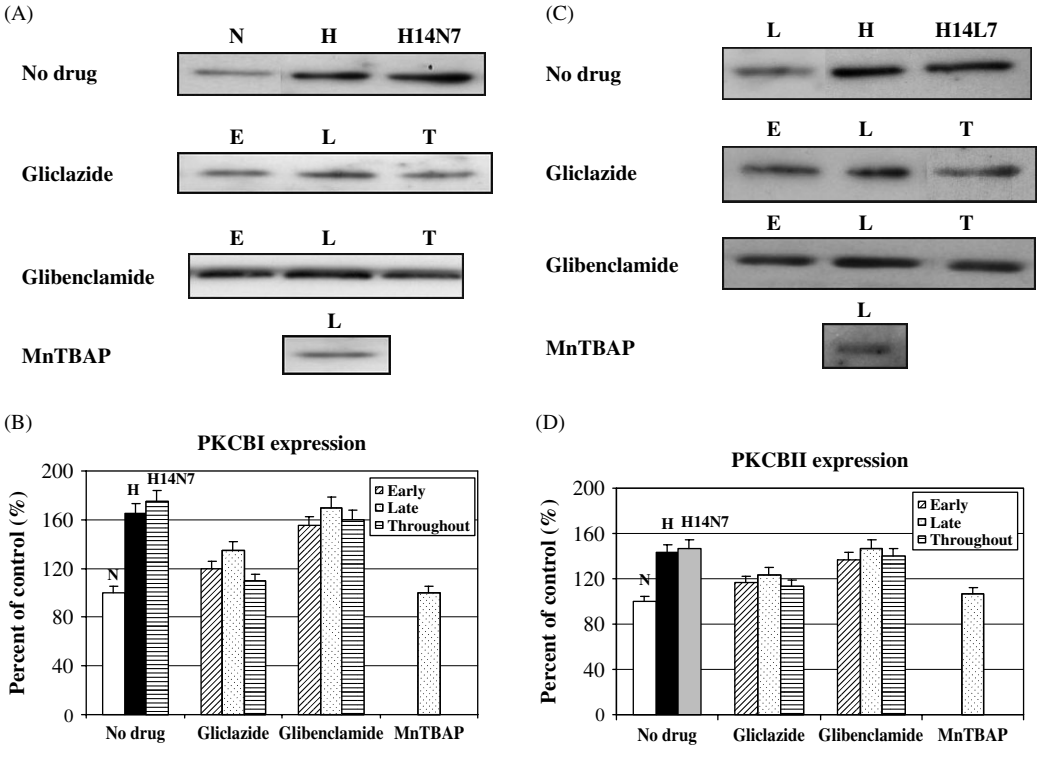

Figure 7. (A) and (C) Representative Western blot of PKCβI and PKBβII isoforms. (D) and (F) Quantification of the signal intensity by densitometry. Bars indicate standard deviation. Cytoskeleton protein actin has been used as a reference

NAD(P)H oxidase components

The expression of the NAD(P)H oxidase sub-components, p22phox and p47phox, increased in cells cultured in high glucose and in memory conditions ($P < 0.001$). Addition of gliclazide produced a comparable reduction in p22phox and p47phox both in the high glucose and in the memory condition ($P < 0.001$), no relevant reduction was observed with glibenclamide (Figure 8). The addition of MnTBAP reduced expression of the NAD(P)H oxidase components to the level observed in the normal glucose-treated control cells ($P < 0.001$).

Discussion

The increased oxidative stress that characterizes diabetes mellitus is considered a major determinant of diabetic endothelial dysfunction [9–12]. In this study, human

DOI: 10.1002/dmrr

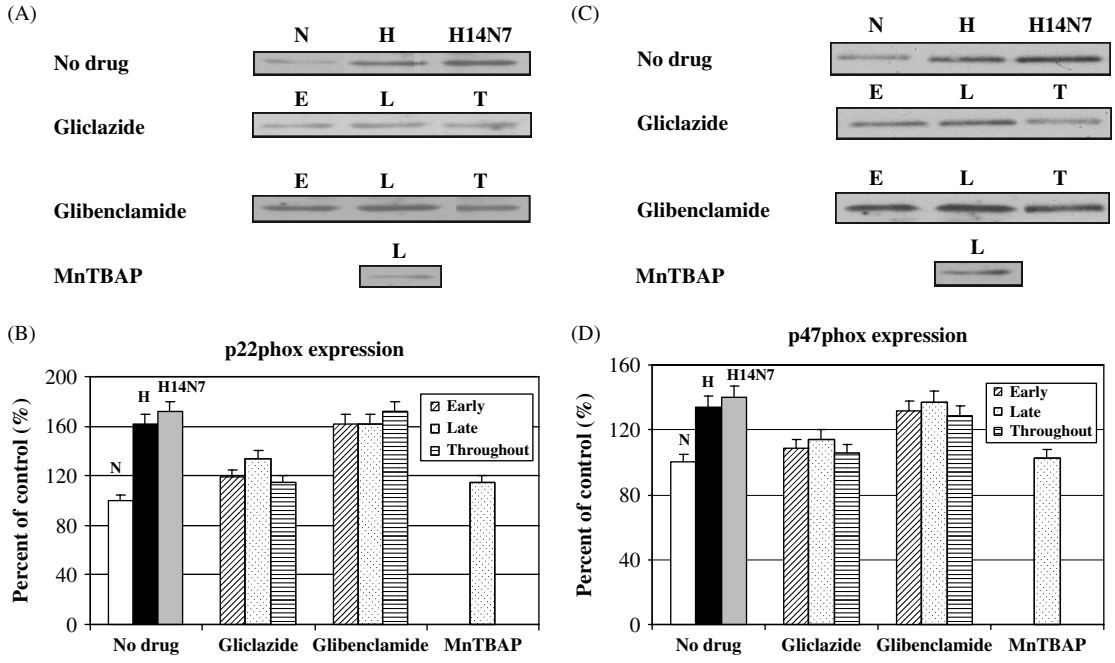

Figure 8. NAD(P)H oxidase expression. (A) and (C) Representative Western blot analysis of p22phox and p47phox expression after 21 days of experiment. (B) and (D) Quantification of the signal intensity by densitometry. Bars indicate standard deviation. Cytoskeleton protein actin has been used as a reference

endothelial cells from umbilical vein were cultured in normal and high-glucose conditions. In agreement with previous studies, exposure of cells to high glucose reduced their viability compared to normal glucose controls [13], stimulated apoptosis [8,14], and resulted in increased expression of fibronectin, a glycoprotein membrane component that is typically increased in the thickened basement membranes of small blood vessels in patients with diabetes [2,15].

Several lines of evidence from our study suggest that hyperglycaemia-induced oxidative stress was responsible for the observed changes in cell viability and function. Cells cultured in high glucose demonstrated increased levels of the antioxidant enzymes MnSOD and catalase, and had larger amounts of nitrotyrosine and 8-OHdG than cells cultured in normal glucose. Nitrotyrosine is considered a marker for the presence of peroxynitrite, a powerful oxidant derived from the reaction of superoxide and nitric oxide as well as of the nitrosilation process.

In vascular cells it is believed that the most important source of ROS is *via* activation of NAD(P)H oxidase [16–18]. We observed an increase in two cytosolic components of NAD(P)H oxidase, p22phox and p47phox, suggesting that high glucose levels may stimulate ROS production through activation of NAD(P)H oxidase in endothelial cells. It is well accepted that activation of PKC is important in the pathogenesis of diabetic complications, and that PKC can activate NAD(P)H oxidase by phophorylation of the p22phox and p47phox subcomponents [19]. Cells exposed to high glucose showed a marked increase in PKC activity, in agreement with other studies [18,20]. Western blot analysis of electrophoresed proteins present in lysed cells after 21 days in culture confirmed that the PKC isoforms, PKC-βI and PKC-βII, which play a key role in most pathophysiologic processes associated with diabetic vascular complications, were increased in the high-glucose media.

In this study we used the already developed experimental model of hyperglycaemic memory by exposing the endothelial cells to 30 mmol/L glucose for 14 days followed by 5 mmol/L glucose for 7 days. Even though cells were exposed to normal glucose for the last 7 days of the experiment, poor cell viability, increased markers of apoptosis and evidence of endothelial dysfunction remained at or near levels observed in cells cultured in continuous high-glucose conditions, suggesting that a cellular 'memory' of hyperglycaemia exists at the level of the endothelial cells.

In the cells cultured in the memory condition, the production of ROS and antioxidant enzymes, expression of NAD(P)H sub-units, PKC activity and expression remained at or above levels observed in cells cultured in continuous high glucose. Treatment with MnTBAP at 14 days was able to reverse these observations confirming our previous report [3] concerning the involvement of oxidative stress in maintaining the memory of the cell for hyperglycaemia-induced damage.

Gliclazide possesses considerable free radical scavenging activity demonstrated both *in vitro* and clinically [21–25]. Importantly, the antioxidant effects of gliclazide are independent of any effects of glycaemic control [6]. In this study, gliclazide was added early (days 1–14), late (days 14–21), or throughout the exposure to the memory condition. Glibenclamide, a sulfonylurea devoid of antioxidant properties, was used for comparative

DOI: 10.1002/dmrr

purposes. The addition of gliclazide was able to attenuate the cellular 'memory' effect of hyperglycaemia in terms of improved cell viability, decreased apoptosis and decreased fibronectin expression while glibenclamide did not show any effect. Addition of gliclazide to the memory condition decreased the production of oxidative stress and antioxidant enzymes, providing further evidence for a role of oxidative stress in maintaining hyperglycaemic memory. Of great relevance, gliclazide was able to reverse the 'memory effect' of hyperglycaemia not only when added late during the experiments in normal glucose, but it was most effective when administered early or throughout 21 days, suggesting that intervention needs to be applied early in the 'disease' to have more chances to be effective in the reversal of hyperglycaemic cellular memory.

Our findings have potential clinical relevance in that, less than optimal glycaemic control during the early years of diabetes appears to have a lasting detrimental effect on the development and progression of complications, even after better glycaemic control is established later in the course of the disease [26]. The concept of hyperglycaemic memory is a further argument for promoting optimal glycaemic control as early as possible after diagnosis [27]. The importance of oxidative stress in this process raises the concept that an antioxidant therapy may offer significant long-term benefits on diabetes-associated vascular complications. An anti-hyperglycaemic agent with possible antioxidant properties, such as gliclazide, may therefore have an enhanced therapeutic role.

Acknowledgements

This study has been supported by an unrestricted grant from the Institute de Recherce Servier, France.

Conflict of interest

None declared.

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