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Original article

Effects of exposure of human islet beta-cells to normal and high glucose levels with or without gliclazide or glibenclamide

S. Del Guerra , V. D'Aleo , R. Lupi , M. Masini , M. Bugliani , U. Boggi , F. Filipponi , P. Marchetti *

Department of Endocrinology and Metabolism, University of Pisa, Ospedale Cisanello, Via Paradisa 2, 56100 Pisa, Italy

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Abstract

Aim. – To evaluate the effects of exposure to high glucose (HG) levels and sulphonylurea on isolated human islet-cell function, and to investigate some of the mechanisms that might be involved.

Methods. – Islet cells were isolated, using collagenase digestion and gradient purification, from 13 pancreata from non-diabetic multiorgan donors (age: 61.2 ± 11.5 years; gender: 7 men/6 women; body mass index: 25.1 ± 2.8 kg/m^2). The cells were then cultured for 5 days with normal glucose (NG) concentrations (5.5 mmol/L), or NG and HG (16.7 mmol/L) levels (alternating every 24 h), with or without the addition of therapeutic concentrations of gliclazide (10 μmol/L) or glibenclamide (1.0 μmol/L). At the end of incubation, functional (glucose-stimulated insulin secretion), morphological (electron microscopy) and molecular (gene and protein expression) studies were performed.

Results. – Insulin secretion differed significantly between study groups, with marked decreases in the presence of HG plus glibenclamide. Compared with NG, insulin expression decreased significantly with HG, and increased similarly with gliclazide as with glibenclamide. However, exposure to gliclazide, but not glibenclamide, significantly induced expression (at both gene and protein levels) of PDX-1, a fundamental beta-cell differentiation transcription factor, and Ki67, a marker of proliferation. However, gliclazide and glibenclamide did not differ in terms of effects on gene expression of the antiapoptotic molecule Bcl2 (increased significantly with both) and the proapoptotic molecule Bax (decreased significantly with both).

Conclusion. – Gliclazide and glibenclamide have different effects on the changes induced by prolonged exposure of human islet cells to high levels of glucose.

Keywords: Pancreatic islets; Gliclazide; Glibenclamide; PDX-1; Ki67

Résumé

Exposition intermittente de cellules β insulaires humaines à des concentrations élevées en glucose. Effets du gliclazide et du glibenclamide.

Objectif. – Évaluer les interactions entre une exposition intermittente à des concentrations élevées en glucose et des sulfonylurées sur les différentes fonctions d'îlots humains isolés et étudier certains des mécanismes impliqués.

Méthodes. – Treize pancréas de donneurs multi-organes non diabétiques (7 M/6F ; âge : 61,2 ± 11,5 ans : IMC : 25,1 ± 2,8 kg/m^2) ont été utilisés. L'isolation des îlots a été réalisée par digestion à la collagénase et gradient de purification Les îlots ont cultivés pendant cinq jours en présence soit de concentrations en glucose stables et normales (NG : 5,5 mmol/L), soit d'une alternance toutes les 24 heures de concentrations en glucose normales et élevées (HG : 16,7 mmol/L), en présence ou non de concentrations thérapeutiques de gliclazide (10 μmol/L) ou de glibenclamide (1,0 μmol/L). En fin d'incubation, ont été réalisées des études fonctionnelles (insulinosécrétion stimulée par le glucose), morphologiques (étude en microscopie électronique) et moléculaires (expression des gènes et des protéines).

Résultats. – La sécrétion d'insuline était significativement différente entre les groupes, avec une diminution plus marquée en présence de HG plus glibenclamide. En comparaison avec NG, l'expression de l'insuline a diminué significativement en présence de HG, et a augmenté de manière similaire avec le gliclazide et le glibenclamide. La culture en présence de gliclazide, mais non de glibenclamide, a induit de manière significative l'expression (à la fois du gène et de la protéine) de PDX-1, facteur fondamental de transcription et différenciation des cellules β, et de Ki67, marqueur

* Corresponding author.
E-mail address: marchant@immr.med.unipi.it (P. Marchetti).

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de prolifération. Il n'y avait pas de différence entre gliclazide et glibenclamide concernant l'expression du gène de la molécule anti-apoptotique Bcl2 (augmentation significative avec les deux sulfonylurées) ni de la molécule pro-apoptotique Bax (diminution significative avec les deux).

Conclusion. – Le gliclazide et le glibenclamide ont des effets différents sur les modifications induites par une exposition prolongée de cellules d'îlot humaines à des concentrations élevées en glucose.

Mots clés : Îlots pancréatiques humains ; Gliclazide ; Glibenclamide ; PDX-1 ; Ki67

1. Introduction

Type 2 diabetes mellitus is the most common form of diabetes, and its prevalence and associated morbidity continue to increase worldwide [1]. Several algorithms and treatment regimens have been proposed to achieve a reasonable degree of glycaemic control and to prevent—or at least delay—the onset of diabetic vascular complications [2–4]. Gliclazide, a second-generation sulphonylurea, is widely used in the treatment of type 2 diabetes [5,6]. The molecule reduces blood glucose levels by increasing insulin secretion from pancreatic beta-cells through interaction with the sulphonylurea receptor (SUR1) of the KATP channel [7]. This interaction is characterized by high affinity and strong selectivity [8,9]. Because of the presence of an aminoazabicyclo-octyl ring in its chemical structure, gliclazide also has antioxidant properties that are independent of its effects on glucose, which might explain the beneficial effects of the drug beyond its antihyperglycaemic activity [10–13].

Gliclazide appears to have a direct beneficial action on pancreatic beta-cells. It has been reported that gliclazide can protect beta-cells from the deleterious effects of reactive oxygen species [14]. The present authors have shown that the molecule can counteract, at least in part, the damage to isolated human pancreatic islet cells caused by prolonged exposure to high glucose (HG) levels, and that the beneficial action of the drug at the beta-cell morphological and survival levels was associated with reduced oxidative stress [15]. The present study investigated the additional molecular mechanisms involved in gliclazide action, focusing in particular on beta-cell ultrastructural features and genes, as well as on the protein expression of molecules involved in beta-cell insulin secretion and turnover.

2. Materials and methods

2.1. Islet preparation and culture

Islet cells were isolated by collagenase digestion and density gradient purification, as described elsewhere [15–17], from the pancreata of 13 human multiorgan donors (age: 61.2 ± 11.5 years; gender: 7 men/6 women; body mass index: 25.1 ± 2.8 kg/m^2), according to protocols approved by our local ethics committee. Aliquots containing approximately 100 islet cells were incubated for 5 days under the four following conditions:

(1) control M199 medium containing normal (5.5 mmol/L) glucose (NG);
(2) medium containing NG or HG (16.7 mmol/L), alternating every 24 h;
(3) medium as in (1) and (2), but with the addition of 10 μmol/L of gliclazide;
(4) medium as in (1) and (2), but with the addition of 1 μmol/L of glibenclamide.

All sulphonylurea concentrations used were in the therapeutic range [18,19]. The use of intermittent HG was based on the assumption that such a condition would better represent the fluctuating glucose concentrations seen in vivo in diabetic patients, as already discussed and applied elsewhere [20]. At the end of the incubation period, functional, morphological and molecular studies were performed as described below.

2.2. Insulin-secretion studies

In these studies [15–17], islet cells were initially kept at 37 °C for 45 minutes in Krebs–Ringer bicarbonate (KRB), with 0.5% (vol/vol) albumin, pH 7.4 and containing 3.3 mmol/L of glucose (washout phase). The medium was then replaced with KRB containing 3.3 mmol/L of glucose to assess basal insulin secretion (45 minutes), followed by a further 45 minutes incubation with 16.7 mmol/L of glucose to assess insulin response to the acute challenge. Insulin was quantified using an immunoradiometric assay (Pantec Forniture Biomediche, Turin, Italy).

2.3. Electron-microscopy evaluation

Electron-microscopy studies, as well as morphological and morphometric analyses, were performed as described elsewhere [15–17]. Insulin granules were identified by their dense cores and white haloes [17]. Volume-density (VD) values were derived from evaluations of 3–5 different islet cells from each pancreas, with 12 photomicrographs taken of each cell at an original magnification of × 7000. Negatives were printed and enlarged to a final magnification of × 16,000. The cytoplasm was used as the reference area. A graticule (11 × 11 cm) composed of 169 points was superimposed on the photomicrographs to count the number of points that intersected with the insulin granules. The VD of the granules was then calculated according to the formula VD = Pi/Pt, where Pi is the number of points within the subcellular component and Pt is the total number of points, expressed in mL/100 mL of tissue (mL%) [15–17].

2.4. Molecular studies

Messenger RNA expression of several genes was measured by reverse transcription polymerase chain reaction (RT-PCR), followed by real-time quantitative RT-PCR. The procedure used