European Journal of Pharmacology 800 (2017) 57–62

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and cellular pharmacology

# Hypoglycemic drugs induce antioxidant aldehyde dehydrogenase activity and remain high in patients with glycemic control in type 2 diabetes

Alejandra Picazo[a], Angélica S. Jiménez-Osorio[b], Porfirio Zúñiga-Mejía[c], José Pedraza-Chaverri[b], Adriana Monroy[d], M. Eunice Rodríguez-Arellano[e], Diana Barrera-Oviedo[a,*]

[a] Faculty of Medicine, Department of Pharmacology, National Autonomous University of Mexico (UNAM), University City 04510, Mexico City, Mexico
[b] Faculty of Chemistry, Department of Biology, National Autonomous University of Mexico (UNAM), University City, 04510 Mexico City, Mexico
[c] Medical Center Dalinde, Tuxpan 25, Roma Sur, 06760 Mexico City, Mexico
[d] General Hospital of Mexico, Dr. Balmis 148, Doctores, 06726 Mexico City, Mexico
[e] Research Department, Hospital Regional "Lic. Adolfo López Mateos", ISSSTE, Av. Universidad 1321, Florida, 01030 Mexico City, Mexico

## ARTICLE INFO

*Keywords:*
Aldehyde dehydrogenase
Superoxide dismutase
Thiobarbituric acid reactive substances

## ABSTRACT

The antioxidant system results essential to control and prevent lipid peroxidation due to stress damage in type 2 diabetes. An example is aldehyde dehydrogenase (ALDH), an enzyme that is involved in the detoxification of aldehydes formed during lipid peroxidation. This study was conducted to evaluate ALDH activity and to determine their association with hypoglycemic treatment in type 2 diabetes patients.

The study population consisted of 422 Mexican subjects: a control group and type 2 diabetes patients. Type 2 diabetes patients were re-classified as those with or without hypoglycemic treatment and those with or without glycemic control (according to glycated hemoglobin (HbA1c)). Clinical parameters, antioxidant enzyme activities (ALDH, superoxide dismutase (SOD), catalase and glutathione peroxidase) and oxidative markers (reactive oxygen species and thiobarbituric acid reactive substances (TBARS)) were evaluated.

The activity of antioxidant enzymes and oxidative stress markers were higher in type 2 diabetes patients with hypoglycemic treatment and without glycemic control than control group. The activity of ALDH and SOD remained high in type 2 diabetes patients with moderate glycemic control while only ALDH's remained high in type 2 diabetes patients with tight glycemic control. Increased ALDH and SOD activities were associated with hypoglycemic therapy. TBARS levels were associated with glycemic control.

The persistence of high ALDH and SOD activities in type 2 diabetes patients with glycemic control may be to avoid a significant damage due to the increase in reactive oxygen species and TBARS. It is possible that this new oxidative status prevented the development the classical complications of diabetes.

## 1. Introduction

Oxidative stress is thought to play a key role in the pathogenesis of type 2 diabetes mellitus by impairing insulin secretion or increasing insulin resistance (Araki and Nishikawa, 2010; Robertson, 2009, 2002). Lipid peroxidation generates toxic aldehydes such as malondialdehyde, acetaldehyde, and 4-hydroxy-2-nonenal that can form adducts with proteins, leading to cell damage and organ dysfunction. Thus, aldehydes may contribute to the deleterious consequences of oxidative stress in diabetes (Turk et al., 2002). Lipid peroxidation is decreased through several enzymatic systems like aldehyde dehydrogenases that are able to oxidize aldehydes to their respective organic acid. ALDH is a crucial enzyme responsible for the metabolism or detoxification of acetaldehyde and other toxic aldehydes (Chen et al., 2014).

The American Diabetes Association (ADA) recommends lifestyle modifications and metformin for initial pharmacological therapy of type 2 diabetes (2014). Studies have shown improvement in the disease complications and antioxidant defense with metformin alone or in combination with other drugs (Chakraborty et al., 2011; Esteghamati et al., 2013, 2000). However, due to the progressive nature of the disease, most patients will require the use of a combination pharmacological therapy to reach therapeutic goals. The ADA recommends adding a sulfonylurea or insulin when metformin monotherapy is insufficient to reach or maintain target goals (ADA, 2014, Nathan et al., 2009; Phung et al., 2010). Some hypoglycemic drugs currently

* Correspondence to: Torre de Investigación Sexto Piso, Lab. 5, Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), C.P. 04510, México DF, Mexico.
*E-mail address:* dianabarrera@hotmail.com (D. Barrera-Oviedo).

http://dx.doi.org/10.1016/j.ejphar.2017.02.027
Received 14 December 2016; Received in revised form 15 February 2017; Accepted 15 February 2017
Available online 16 February 2017
0014-2999/ © 2017 Elsevier B.V. All rights reserved.

employed (insulin, sulfonylureas or biguanidines) improve antioxidant defense and prevent diabetes complications (ADA, 2014, Araki and Nishikawa, 2010, Esteghamati et al., 2013; Shen, 2012).

In this observational study, the effects of hypoglycemic drugs on ALDH (EC 1.2.1.3) activity were explored in type 2 diabetes patients. Moreover, the levels of the widely studied oxidative stress status markers (reactive oxygen species and TBARS) as well as the antioxidant enzymes (SOD (EC 1.15.1.1), CAT (EC 1.11.1.6) and GPx (EC 1.11.1.9)) that metabolize the principal reactive oxygen species were determined.

## 2. Materials and methods

### 2.1. Study design and subjects

The present study was conducted according to the Declaration of Helsinki. The project was approved by the Research, Ethics and Biosafety Committee of the Research Department, Hospital Regional "Lic. Adolfo López Mateos", ISSSTE (registration number 209.2013), General Hospital of Mexico (registration number DI/11/UME/4/46) and Faculty of Medicine, UNAM (Project number 059–2012). All participants agreed to participate in the study by signed a consent form prior to the application of the research protocol.

Inclusion criteria were according to ADA standards (ADA, 2014; Nathan et al., 2009): 1) Control subjects (CT): Without any type of diabetes, fast plasmatic glucose ≤99 mg/dl; 2) Patients with type 2 diabetes (D NT): Diagnosis of type 2 diabetes, fast plasmatic glucose ≥126 mg/dl and without treatment; 3) Patients with type 2 diabetes and treatment (D T): Diagnosis of type 2 diabetes and hypoglycemic drugs treatment (insulin, metformin and glibenclamide alone or in combination); 4) Patients with type 2 diabetes treated with hypoglycemic drugs and with glycemic control (D T C): according to HbA1c levels ( < 6.5%); and 5) Patients with type 2 diabetes treated with hypoglycemic drugs and without glycemic control (D T NC): according to HbA1c levels (≥6.5%). D T C and D T NC groups were subdivided into two groups for analysis.

Exclusion criteria of subjects were as follows: with type 1 diabetes, gestational diabetes, pregnant or lactating women, no Mexican origins, younger than 35 years old, alcoholics or with excessive alcohol consumption, with bleeding disorders, erythrocythemia or anemia, with diabetic nephropathy, with mental health problems or unable to sign the informed consent to participate in the study. Only subjects with complete data were included for the statistical analysis.

### 2.2. Sample collection and clinical analysis

Two samples (6 ml each) of venous peripheral blood were obtained from antecubital vein after 8–10 h of nighttime fasting. One sample was collected into serum separator tube and was centrifuged at 1500×G for 10 min at 4 °C. Glucose was measured in serum using a Miura 200 Auto analyzer (Diamond Diagnostics, Holliston MA, USA) employing commercial kit (I.S.E., Freiburg Breisgau, Germany). The other sample tube supplemented with EDTA was used for blood collection. Ten µl of EDTA-blood were used for HbA1c quantification in a Miura 200 Auto analyzer and the remnant was used to obtain plasma for insulin evaluation and for erythrocytes isolation by centrifugation of 1500×G for 10 min at 4 °C. Insulin was determined in an ARCHITECT auto analyzer (Abbot, Lake Forest IL, USA). A homeostasis model assessment (HOMA2) calculator was used to calculate HOMA2-IR (estimates insulin resistance), HOMA-S (estimates insulin sensitivity) and HOMA-B (estimates steady state beta cell function) indices and can be found at the following website: http://www.dtu.ox.ac.uk/index.php?maindoc=/homa/(Walace et al., 2004).

The white phase cells was removed and erythrocytes were washed twice with cold saline solution (154 mmol/l) and hemolysates with double distilled water (1:5 cells-water) and centrifuged at 1500×G for 10 min at 4 °C. Lysates from erythrocytes were used to determine the enzyme activity of ALDH, SOD, CAT and GPx as well as the reactive oxygen species and TBARS levels by spectrophotometric or fluorometric methods.

### 2.3. Activity of antioxidant enzymes in erythrocytes hemolysates

ALDH activity was determined by measuring the generation of NADH at 340 nm (Rawles et al., 1987). The enzyme activity was expressed as U/mg hemoglobin. SOD activity was determined using the reduction of nitroblue-tetrazolium to formazan that is detectable at 586 nm. One unit of SOD was defined as the amount of protein that inhibits NBT reduction by 50% (Oberley and Spitz, 1984). The enzyme activity was expressed as U/mg protein. CAT activity was determined by measuring the consumption of hydrogen peroxide at 240 nm (Aebi, 1984), the enzyme activity was expressed as κ/mg protein. The activity of GPx was determined measuring the consumption of NADPH at 340 nm. One unit of GPx was defined as the amount of enzyme that oxidizes 1 µmol of NADPH/min, the enzyme activity was expressed as U/mg protein (Barrera et al., 2003). The total protein content was determined using the colorimetric method of Lowry et al. (1951).

### 2.4. Oxidative stress markers levels in erythrocytes hemolysates

Reactive oxygen species are formed by the incomplete reduction of oxygen and a fluorometric assay, based on the oxidation of 2-dichlodihydrofluorescein diacetate to 2, 7-dichloro-fluorescein was employed to detect them. Results were expressed in arbitrary fluorescence units. Fluorescent signals were recorded at excitation and emission wavelengths of 488 and 532 nm, respectively (Silva-Adaya et al., 2008). Lipoperoxidation was determined by TBARS assay as products of the polyunsaturated fatty acids decomposition react with thiobarbituric acid. This controlled reaction was quantified spectrophotometrically at 532 nm (Reznick and Packer, 1994).

### 2.5. Statistical analyses

Reduction of the dimension of the data was performed on variables with missing data. Data were analyzed using GraphPad Prism 5.0 (Graph Pad, San Diego, USA). Spearman correlation test, Mann-Whitney test or Kruskal-Wallis test followed by the multiple comparison test proposed by Dunn were used. Data were shown as the median (minimum-maximum) or as mean ± S.E.M. Logistic regression analysis (Tabachnick and Fidell, 2012) was performed using STATA 12 (StataCorp, College Station, TX, USA). Data were shown as Odds Ratio (OR) and 95% confidence interval, $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Clinical characteristics

After applying inclusion and exclusion criteria, 422 subjects were included in the study. The classification of participants was conducted to determine some characteristics of diabetic patients with treatment or without treatment, diabetic patients with glycemic control and diabetic patients without glycemic control. The values are shown in Table 1. The average time from diagnosis of diabetes was 8.0 (0–30) years. Woman predominance (55%) was found in recruited population. Treatment was: glibenclamide (4 patients), insulin (15 patients), metformin (37 patients), glibenclamide/metformin (23 patients), insulin/metformin (45 patients) and glibenclamide/insulin/metformin (2 patients).

### 3.2. Antioxidant enzymes

ALDH, SOD, CAT and GPx activities were significantly higher in D

Download English Version:

https://daneshyari.com/en/article/5554639

Download Persian Version:

https://daneshyari.com/article/5554639

Daneshyari.com