TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2008; 38 (6): 545-548
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E-mail: medsci@tubitak.gov.tr

Effect of Metformin or Gliclazide on Lipid Peroxidation and Antioxidant Levels in Patients with Diabetes Mellitus

Ramazan MEMİŞOĞULLARI[1]
Mehmet TÜRKELİ[2]
Ebubekir BAKAN[3]
Fatih AKÇAY[3]

[1] Department of Biochemistry, Faculty of Medicine, Düzce University, Düzce - TURKEY
[2] Department of Internal Medicine, Faculty of Medicine, Atatürk University, Erzurum - TURKEY
[3] Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum - TURKEY

Aim: The association between oxidative stress and diabetic complications is well known. The main goal of antidiabetic therapy is to prevent the complications of diabetes. Gliclazide (sulfonylurea) and metformin (biguanide) are two oral antidiabetic drugs that have been proven to prevent diabetic complications. In this study, we aimed to investigate the antioxidant effects of gliclazide or metformin.

Materials and Methods: In this study, we evaluated 46 patients with type 2 diabetes mellitus. The patients were divided into three groups according to their treatment modality of gliclazide, metformin, or diet. Erythrocyte glutathione peroxidase (Gpx), glutathione S-transferase (GST), and catalase (CAT) activities and malondialdehyde (MDA) and glutathione (GSH) levels of the patients were measured and compared.

Results: The erythrocyte Gpx, GST, and CAT activities were significantly higher, whereas MDA levels were significantly lower in both gliclazide and metformin groups when compared with the diet group. There was no statistically significant difference between gliclazide and metformin groups in terms of activities of antioxidant enzymes and levels of MDA. In addition, GSH levels were not different among the groups.

Conclusions: The data obtained in this study showed that gliclazide or metformin administration may decrease oxidative stress, and both drugs had similar effects.

Key Words: Diabetes mellitus, gliclazide, metformin, antioxidant enzymes, malondialdehyde

Diyabetli Hastalarda Gliklazid veya Metformin'in Lipid Peroksidasyonu ve Antioksidan Düzeyleri Üzerine Etkisi

Amaç: Oksidatif stress ve diyabetin komplikasyonları arasındaki ilişki iyi bilinir. Antidiyabetik tedavinin temel amacı diyabetin komplikasyonlarından korumaktır. Diyabetin komplikasyonlarından koruduğu kanıtlanmış olan sülfanilüre gliklazid ve biguanid metformin iki oral antidiyabetik ilaçtır. Bu çalışmada gliklazid veya metformin'in antioksidan özelliklerini araştırmayı amaçladık.

Yöntem ve Gereç: Bu çalışmada 46 tip 2 diyabetli hasta değerlendirildi. Hastalar gliklazid, metformin veya sadece diyet almalarına göre 3 gruba ayrıldı. Eritrosit glutatyon peroksidaz (Gpx), glutatyon S-transferaz (GST) ve katalaz (CAT) aktiviteleri, lipid peroksidasyon göstergesi olarak malondialdehid (MDA) ve glutatyon (GSH) düzeyleri ölçüldü ve karşılaştırıldı.

Bulgular: Sadece diyet alan grupla karşılaştırıldığında hem gliklazid hem de metformin grubunda eritrosit Gpx, GST ve CAT aktiviteleri anlamlı olarak daha yüksekken, MDA düzeyi ise anlamlı olarak daha düşüktü. Antioksidan enzim aktiviteleri ve MDA ile GSH düzeyleri açısından gliklazid ve metformin grupları arasında istatistiksel anlamlı bir fark yoktu.

Sonuç: Çalışmadan elde edilen veriler gliklazid veya metformin tedavisinin oksidatif stresi azaltabileceğini ve bu etkinin gliklazid ve metformin gruplarında benzer olduğunu göstermiştir.

Anahtar Sözcükler: Diabetes Mellitus, gliklazid, metformin, antioksidan enzimler, malondialdehid

Received: September 21, 2007
Accepted: August 27, 2008

Correspondence
Ramazan MEMİŞOĞULLARI
Department of Biochemistry, Faculty of Medicine, Düzce University, Düzce - TURKEY

rmemisogullari@hotmail.com

Introduction

It is well known that oxidative stress plays important roles in the pathophysiology of many diseases such as diabetes, Behçet's disease, and rheumatoid arthritis, and impairs various cellular functions (1-3). To control the flux of reactive oxygen species (ROS), aerobic cells have antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (Gpx), catalase (CAT), glutathione S-transferase (GST), as well as non-enzymatic antioxidants such as glutathione (GSH) and vitamins C and E to defend themselves against oxidative stress. SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide (H_2O_2). H_2O_2 can be transformed into H_2O and O_2 by CAT

or into H_2O by Gpx. Gpx is a selenoprotein, which reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing GSH (1-4).

Gliclazide and metformin have different mechanisms to reduce glycemia. Gliclazide, a second-generation sulfonylurea that possesses a unique azabicyclo-octyl ring, is reported to act as a general free radical scavenger *in vitro* (5-8). It has also been reported that metformin has an antioxidant activity (7-12). In our literature search, although there are a few reports about the antioxidant effects of gliclazide or metformin separately, we could not find any human study evaluating both gliclazide and metformin on the basis of lipid peroxidation and antioxidant enzymes, or comparing them with respect to their effect on antioxidant and oxidant parameters. Thus, we planned to evaluate both gliclazide and metformin on the basis of lipid peroxidation and antioxidant enzymes.

Materials and Methods

In this study, we evaluated 46 patients with type 2 diabetes mellitus. The patients were divided into three groups according to their treatment modalities. Sixteen patients were administered gliclazide, 15 patients were treated with metformin and 15 patients received only diet treatment. Mean duration of diabetes in these groups was 12.2 ± 0.24, 13.4 ± 0.39 and 11.3 ± 0.27 (mean ± SE) years, respectively. The patients selected for diet therapy were those who discontinued their therapy against physician advice or who rejected any medication. None of the patients was a smoker. None consumed alcohol, received any medication except gliclazide or metformin, or had any other chronic disease. Erythrocyte Gpx, GST, and CAT activities and malondialdehyde (MDA), as a lipid peroxidation marker, and GSH levels of the patients were measured and compared.

Biochemical Measurements

After the collection of blood samples into tubes containing EDTA, erythrocytes were washed 3 times with serum physiologic (0.9% NaCl). Erythrocyte sediments were prepared for the determination of Gpx, GST, and CAT activities and MDA and GSH levels. Erythrocytes were then hemolyzed with diluted deionized water (50-fold), and the analyses were carried out on this hemolyzed supernatant fraction. Hemoglobin (Hb) values were measured with a GEN-S counter (Beckman-Coulter, USA). Hemolyzed samples were kept at –80°C until biochemical determinations.

Venous blood was also collected in vacutainers without additive for routine biochemical determination, allowed to clot for 30 min at room temperature, and centrifuged at 3000 x g for 5 min to obtain serum. Serum aliquots were stored at -80°C until biochemical analyses.

Serum glucose (GLU) levels were measured with an autoanalyzer (Hitachi 717 Analyser, Hitachi, Japan) using commercial kits. Hemoglobin A1C (HbA1C) levels were measured by high performance liquid chromatographic assay (Hi-Auto A1C HA 8121; Kyoto Dai-ichi Kagaku, Japan).

Gpx activity was measured according to the Paglia and Valentina method (13). Gpx activity was expressed as U/g Hb. CAT activity was measured in hemolysates at 20°C, according to the method of Aebi (14). Using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}, the rate of the first 30 s was used to calculate the activity. CAT activity was expressed as U/mg Hb. Virtually all of the non-protein sulfhydryl groups of erythrocytes are in the form of reduced GSH. 5,5'- Dithio bis (2-nitrobenzoic acid) is a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration (15). GSH activity was expressed as mmol/g Hb. GST activity of the supernatant was measured by using 1-chloro-2,4-dinitrobenzene (16). The activity was expressed as U/g Hb. MDA was determined on the basis of spectrophotometric absorbance measurement of the pink-colored product of the thiobarbituric acid reactive substance (TBARS) complex (17). Total TBARS was expressed as MDA. Results are expressed as nmol/g Hb. These measurements were carried out using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

Statistical Analysis

The findings were expressed as the means ± SE (standard error). Firstly, the data were analyzed with Kolmogorov-Smirnov test for parametric or non-parametric discrimination. The data showed parametric distribution according to this test. Statistical analyses were undertaken using one-way ANOVA. LSD (least significant difference) multiple range test was used to compare the mean values (acceptable significance was recorded when p values were <0.05). Correlation analyses were undertaken using Pearson's rank correlation test. Statistical analysis was performed with

Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

Results

Comparisons of the groups are shown in Table . There was no statistically significant difference among groups in terms of GLU, HbA1C, and body mass index (BMI). As shown in Table , the erythrocyte Gpx, CAT, and GST activities were significantly higher; in contrast, MDA levels were significantly lower in both gliclazide and metformin groups than in the diet group (Table). There was no statistically significant difference between gliclazide and metformin groups in terms of activities of antioxidant enzymes and levels of MDA. In addition, GSH levels did not differ between the groups.

In correlation analysis, there was a negative correlation between GSH and GLU level ($r = -0.65$; $P < 0.01$) in the metformin group. A positive correlation between MDA and HbA1C ($r = 0.75$; $P < 0.001$) and a negative correlation between Gpx and MDA level ($r = -0.54$; $P < 0.05$) were determined in the diet group.

Discussion

The data obtained in this study showed that gliclazide or metformin had an oxidative stress-decreasing effect, apart from their anti-hyperglycemic effect. This effect was similar in both metformin and gliclazide groups.

In previous studies, free radical scavenging properties of gliclazide were reported and these properties are not shared by other sulfonylureas, such as glibenclamide or tolbutamide (5,18). In type 2 diabetes, gliclazide has been reported to reduce *in vitro* low-density lipoprotein (LDL) oxidation and to reduce both endothelial and monocyte cell-mediated LDL oxidation and monocyte adhesion to endothelial cells *in vitro* (5-7).

Metformin is a biguanide antihyperglycemic agent used for the management of type 2 diabetes. Several studies have shown reduced cardiovascular-related mortality rates in metformin-receiving patients. These observations suggest that metformin might have an additional mechanism of action beyond its antihyperglycemic properties (7-11,19). Khouri et al. (20) reported that metformin does not scavenge $O^{\cdot}_2$ radicals, but is able to react with $^{\cdot}OH$ radical. Their results obtained with an *in vitro* model allow assuming that metformin, at a molecular level, is not a very good scavenger of ROS. Consequently, it seems that metformin would certainly exert its *in vivo* antioxidant activity by different pathways other than the simple free radical scavenging action, such as increasing the antioxidant enzyme activities, decreasing the markers of lipid peroxidation and inhibiting the formation of advanced glycation end products (20).

Measurement of TBARS is probably the most widely used assay in biochemical studies. Alper et al. (21) reported that gliclazide treatment alone decreased liver tissue MDA level significantly; however, it was unable to decrease plasma MDA levels. We have found that MDA levels were lower in both the gliclazide and metformin groups than in the diet group.

Table. Results of study groups (mean ± SE).

	Gliclazide Group n = 16	Metformin Group n = 15	Diet Group n = 15
Gpx ($Ug^{-1}Hb$)	41.13 ± 1.95[a]	39.51 ± 1.94[a]	29.20 ± 1.99
CAT ($Umg^{-1}Hb$)	2.44 ± 0.06[a]	2.57 ± 0. 10[a]	2.09 ± 0.16
GSH ($mmolg^{-1}Hb$)	8.95 ± 0.48	8.49 ± 0.87	8.29 ± 0.58
GST ($Ug^{-1}Hb$)	26.81 ± 1.6[a]	31.67 ± 2.58[a]	15.87 ± 2.71
MDA ($nmolg^{-1}Hb$)	11.5 ± 0.23[a]	11.5 ± 0.33[a]	13.2 ± 0.55
GLU (mg/dL)	143.8 ± 11.5	147.3 ± 12.6	160.8 ± 15.3
HbA1C (%)	7.5 ± 0.3	7.3 ± 0.4	8.2 ± 0.2
BMI (kg/m^2)	26.9 ± 1.2	27.1 ± 0.8	26.7 ± 1.0

[a]: $P < 0.05$, when compared with diet group

Gpx: Glutathione peroxidase. CAT: Catalase. GSH: Glutathione. GST: Glutathione S- transferase. MDA: Malondialdehyde. GLU: Glucose. HbA1C: Hemoglobin A1C. BMI: Body mass index.

The levels of erythrocyte antioxidants such as GSH, Gpx, GST, and CAT tend to decrease, and MDA tends to increase in diabetic patients (2). In our study, Gpx, GST, and CAT activities were higher in the gliclazide and metformin groups than in the diet group. Although GSH levels were higher in both metformin and gliclazide groups, this was not statistically significant when compared with the diet group. That is to say, antioxidant effects of gliclazide are similar to those of metformin. Pavlovic et al. (22) reported that metformin monotherapy decreased erythrocyte MDA levels and increased erythrocyte CAT activity and GSH level. However, we could not determine higher GSH levels with metformin therapy. We found that there was a positive correlation between MDA and HbA1C level and a negative correlation between MDA level and Gpx activity in the diet group.

ROS have been reported to be affected with better glycemic control (2,3). However, there was no statistical difference between the groups in terms of glycemic control. Therefore, the results of this study could be interpreted as independent from the effect of glycemic control. Nevertheless, we think that the subject still requires further studies with larger cohorts before reaching a final decision.

References

1. Taysi S, Kocer I, Memisogullari R, Kiziltunc A. Serum oxidant/antioxidant status in patients with Behçet's disease. Ann Clin Lab Sci 2002; 32: 377-82.
2. Memisogullari R, Taysi S, Bakan E, Capoglu I. Antioxidant status and lipid peroxidation in type II diabetes mellitus. Cell Biochem Funct 2003; 21: 291–6.
3. Memisogullari R, Bakan E. Levels of ceruloplasmin, transferrin, and lipid peroxidation in the serum of patients with type 2 diabetes mellitus. J Diabetes Complications 2004; 18: 193– 7.
4. Memisogullari R, Gümüştekin K, Dane Ş, Akçay F. Effect of preinjury supplementation of selenium or vitamin E on lipid peroxidation and antioxidant enzymes in burn injury. Turk J Med Sci 2006; 36: 141-6.
5. Kimoto K, Suzuki K, Kizaki T, Hitomi Y, Ishida H, Katsuta H et al. Gliclazide protects pancreatic b-cells from damage by hydrogen peroxide. Biochem Bioph Res Co 2003; 303: 112–9.
6. Jennings PE, Belch JJ. Free radical scavenging activity of sulfonylureas: a clinical assessment of the effectiveness of gliclazide. Ter Arkh 2001; 73: 27-31.
7. Tessier D, Maheux P, Khalil A, Fulop T. Effects of gliclazide versus metformin on the clinical profile and lipid peroxidation markers in type 2 diabetes. Metabolism 1999; 48: 897-903.
8. Sliwinska A, Blasiak J, Kasznicki J, Drzewoski J. In vitro effect of gliclazide on DNA damage and repair in patients with type 2 diabetes mellitus (T2DM). Chem Biol Interact 2008; 173: 159-65.
9. Formoso G, De Filippis EA, Michetti N, Di Fulvio P, Pandolfi A, Bucciarelli T et al. Decreased in vivo oxidative stress and decreased platelet activation following metformin treatment in newly diagnosed type 2 diabetic subjects. Diabetes Metab Res Rev 2008; 24: 231-7.
10. Majithiya JB, Balaraman R. Metformin reduces blood pressure and restores endothelial function in aorta of streptozotocin-induced diabetic rats. Life Sci 2006; 78(22): 2615-24.
11. Iida KT, Kawakami Y, Suzuki M, Shimano H, Toyoshima H, Sone H et al. Effect of thiazolidinediones and metformin on LDL oxidation and aortic endothelium relaxation in diabetic GK rats. Am J Physiol Endocrinol Metab 2003; 284: E1125–E1130.
12. Bonnefont-Rousselot D. Antioxidant and anti-AGE therapeutics: evaluation and perspectives. J Soc Biol 2001; 195: 391-8.
13. Paglia DE, Valentina WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158–69.
14. Aebi H. Catalase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. Weinheim: Verlag Chemie; 1974. pp. 673–8.
15. Panteghini M, Bais R, van Solinge WW. Methodology, Enzymes. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Philadelphia: WB Saunders; 2006. pp. 1990–2106.
16. Sen CK, Marin E, Kretzschmar M, Hänninen O. Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. J Appl Physiol 1992; 73: 1265–72.
17. Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 1989; 38: 1539–43.
18. Vallejo S, Angulo J, Peiro C, Sanchez-Ferrer A, Cercas E, Llergo JL et al. Prevention of endothelial dysfunction in streptozotocin-induced diabetic rats by gliclazide treatment. J Diabetes Complications 2000; 14: 224-33.
19. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother 2005; 59: 365–73.
20. Khouri H, Collin F, Bonnefont-Rousselot D, Legrand A, Jore D, Gardès-Albert M. Radical-induced oxidation of metformin. Eur J Biochem 2004; 271: 4745–52.
21. Alper G, Irer S, Duman E, Çağlayan O, Yılmaz C. Effect of l-deprenyl and gliclazide on oxidant stress/antioxidant status and DNA damage in a diabetic rat model. Endocr Res 2005; 31: 199–212.
22. Pavlovic D, Kocic R, Kocic G, Jevtovic T, Radenkovic S, Mikic D et al. Effect of four-week metformin treatment on plasma and erythrocyte antioxidative defense enzymes in newly diagnosed obese patients with type 2 diabetes. Diabetes Obes Metab 2000; 2: 251-6.