Kafkas Univ Vet Fak Derg
17 (2): 235-238, 2011
DOI:10.9775/kvfd.2010.3071

RESEARCH ARTICLE

The Effect of Glurenorm (Gliquidone) on Aorta in STZ Induced Diabetic Rats

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Makale Kodu (Article Code): KVFD-2010-3071

Summary

Vascular diseases are one of the common complications of diabetes mellitus. In diabetes, increased free radical formation raises the incidence of atherosclerosis and cardiovascular diseases. Regardless of the type of diabetes, the objective of the therapy is to achieve normoglycemia and to prevent or delay the complications. Glurenorm (gliquidone) is one of the members of sulphonylurea group oral antidiabetics. Sulphonylurea oral antidiabetics act via stimulating insulin release from β cells of the pancreas. Therefore, the aim of the study was to investigate the effect of glurenorm (gliquidone, 10 mg/kg) on the aorta of streptozotocin-induced diabetic rats in terms of nonenzymatic glycation, lipid peroxidation and reduced glutathione. Both diabetic and control group rats have taken the drug daily, until the end of the experiment, at day 42. Blood samples and aorta was taken from each rat at day 42. Blood glucose was measured by o-toluidine method. Glurenorm decreased the blood glucose and increased the body weights of diabetic rats. However, glurenorm did not decrease non-enzymatic glycation of aorta proteins in diabetic rats, but it decreased lipid peroxidation of aorta. Although the aorta lipid peroxidation level was decreased in glurenorm given diabetic rats, glutathione level did not increase. This may show that oxidative damage continues during the glurenorm treatment. Glurenorm also did not change the electrophoretic pattern of aorta proteins. The results indicate the usage of glurenorm is effective on decreasing of blood glucose but not on decreasing nonenzymatic glycation or oxidative damage in aorta samples of diabetic rats.

Keywords: *Diabetes, Aorta, Glurenorm, Nonenzymatic glycation, Lipid peroxidation, Glutathione*

STZ ile Diyabet Oluşturulan Sıçanlarda Glurenormun (Gliquidone) Aort Üzerine Etkisi

Özet

Vasküler hastalıklar diyabetin yaygın komplikasyonlarından biridir. Diabette artan serbest radikal oluşumu ateroskleroz ve kardiyovasküler hastalıkların insidansını arttırır. Diyabetin tipinden bağımsız olarak tedavinin amacı, kan şekerini normal düzeyde tutarak komplikasyonları önlemektir. Glurenorm (gliquidone) sülfonilüre grubu antidiyabetiklerin bir üyesidir. Sülfonil üre oral antidiyabetikleri pankreasın beta hücrelerinden insulin çıkışını uyararak etki ederler Bu nedenle, çalışmanın amacı streptozotosin (STZ) ile diyabet oluşturulan sıçanların aortları üzerine glurenormun (gliquidone, 10 mg/kg) etkisini nonenzimatik glikasyon, lipid peroksidasyon ve indirgenmiş glutatyon açısından incelemektir. Diyabet ve kontrol gruplarındaki sıçanların hepsine deneyin sonu olan 42. güne kadar hergün ilaç verildi. 42. günde her sıçanın kan örnekleri ve aortları alındı. Kan glukozu o-toluidine metodu ile ölçüldü. Glurenorm diyabetik sıçanların kan şekerini düşürdü ve vücut ağırlıklarını arttırdı. Bununla birlikte, glurenorm diyabetik sıçanların aort proteinlerinin nonenzimatik glikasyonunu azaltmadı fakat lipid peroksidasyon seviyelerini azalttı. Glurenorm verilen diyabetik sıçanlarda lipid peroksidasyonu azalmasına rağmen glutatyon miktarı artmadı. Bu da oksidatif hasarın devam ettiğini göstermektedir. Glurenorm ayrıca aort proteinlerinin elektroforetik modelini de değiştirmedi. Bu sonuçlar glurenormun diyabetik sıçanların kan şekerini düşürmede etkili olduğu ancak aort dokusundaki nonenzimatik glikasyonu ve oksidatif hasarı azaltmadığını göstermektedir.

Anahtar sözcükler: *Diyabet, Aort, Glurenorm, Nonenzimatik glikasyon, Lipid peroksidasyon, Glutatyon*

INTRODUCTION

Hyperglycemia is a major risk factor responsible for the development and progression of vascular complications of diabetes. Several hyperglycemia-induced mechanisms may induce vascular dysfunctions that include increased

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polyol pathway flux, altered cellular redox state, increased formation of diacylglycerol and the subsequent activation of protein kinase C [1]. There is growing evidence that diabetes is associated with vascular complications, which could be due to oxidative stress coupled to increased production of reactive oxygen species [2]. Results from human and animal studies show that free radical mediated oxidative stress play an important role in cardiac dysfunction in diabetes. The increased production of oxygen species is often associated with compromised natural antioxidant defense systems in diabetic tissues. In response to oxidative stress, antioxidant systems are believed to be induced to protect cellular functions, which maintain in vivo homeostasis [3].

Treatment of diabetes mellitus (DM) is essential for preventing the diabetic complications. Sulphonylurea oral antidiabetics are thought to mainly act via stimulating insulin release from β cells of the pancreas. Only 5% of the metabolites are eliminated via the kidneys and the remainder via the bilary tract [4,5]. Glurenorm (gliquidone) is one of the members of sulphonylurea group oral antidiabetics. It decreases the K^+ permeability of the β-cell membrane, thereby causing a depolarization. This activates voltage-dependent calcium channels, permitting Ca^{2+} influx, which eventually stimulates insulin release [4,5]. To the best of our knowledge, this is the first report showing the effects of glurenorm (gliquidone, 10 mg/kg) on aorta oxidant system in diabetes. The aim of the study, therefore, was to investigate the effect of glurenorm on aorta in terms of nonenzymatic glycation (NEG), lipid peroxidation (LPO) and reduced glutathione (GSH) in streptozotocin (STZ)-induced diabetic rats.

MATERIAL and METHODS

Animals and Treatment

43 female Swiss Albino rats, 6 to 6.5 months old, were used. They were divided into four groups; control, control + glurenorm, diabetic and diabetic + glurenorm. The rats in diabetic groups were fasted 18 h and rendered diabetic by a single intraperitoneal injection of 65 mg kg^{-1} STZ (Sigma) in a freshly prepared citrate buffer (pH = 4.5). Fourteen days later, glurenorm (gliquidone, Eczacıbaşı, Turkey, 10 mg kg^{-1}) was given by gavage technique daily until the end of the experiment at day 42. On day 42 blood samples and aorta tissue samples were taken from all rats under ether anesthesia. All experiments were carried out in accordance with the guidelines of the Animal Care and Use Committee of Istanbul University experimental Medical Research and Application Institute (DETAE).

Aort Tissue Sampling and Homogenization

Rats were submitted to a laparotomy for exsanguination via abdominal aorta and to a thoracotomy for thoracic aorta harvesting. The thoracic aorta was carefully dissected free of connective tissue and immediately immersed in a saline solution. After washing the aorta tissue with saline solution, the tissue was dried and weighed. It was put in a glass homogenizer cooled with ice. Aorta tissue samples of each rat were homogenized in saline solution to obtain 10% homogenate.

Biochemical Analysis

Fasting Blood Glucose

Fasting blood glucose was determined by o-toluidine method [6].

Aorta Tissue Total Protein Levels and SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Aorta tissue total protein level was determined by the method of Lowry [7], using bovine serum albumin as a standard, reading absorbance at 500 nm and expressing the total protein level in mg protein g^{-1}aorta. Electrophoretic examination of aorta proteins was carried out by SDS-PAGE [8].

Aorta Tissue Lipid Peroxidation and Reduced Glutathione Levels

The MDA levels were measured by the method of Ledwozyw et al.[9] for products of lipid peroxidation. Results were expressed as nmol MDA mg^{-1} protein. GSH was determined by the spectrophotometric method using Ellman's reagent [10] and the results were expressed as mg GSH mg^{-1} protein.

Aorta Tissue Protein Nonenzymatic Glycation

Aorta tissue protein glycation was asssed by the 2-thiobarbituric acid method [11]. The assay involved hydrolyzing each 0.5 mL homogenate with 0.5 mL of 0.5 M oxalic acid in an autoclave for 1 h 124±1°C. To this, 0.5 mL 40% trichloracetic acid (w/v) was added, mixed, centrifuged at 1500 x g for 10 min, and filtered using filter paper. Absorbance at 443 nm was recorded. Then, 0.75 mL of supernatant was incubated in 0.25 mL of 0.05 M 2-thiobarbituric acid at 37°C for 30 min. After standing for 15 min at room temperature, absorbance was again measured at 443 nm and the differences between the first and second absorbances were calculated. The protein glycation values were expressed as nmol fructose mg^{-1} protein. Commercial fructose (Sigma) was used as a standard.

Statistical Analysis

The results were evaluated using ANOVA variance analysis and student t-test [12,13] using SPSS 11.5 statistical package programme.

RESULTS

Prior to inducing diabetes (day 0), the groups were

checked for the differences in weight and blood glucose, but none were found. The mean levels of blood glucose and body weight for the four groups are shown in *Table 1*.

In morphological evaluation aorta tissue samples from the control group showed normal vascular morphology. However the thickness of aorta tissue samples were decreased in diabetic group. Glurenorm administration to the diabetic group did not induce any morphological change in the thickness of aorta tissue samples.

Table 2 shows the values and differences between groups for aorta tissue total protein, NEG, LPO, GSH at the end of the experiment. Although macroscopic analysis revealed no damage of rat aortas in any groups, NEG in diabetic group was significantly higher than controls (control and control + glurenorm) in both diabetic groups (diabet, diabet + glurenorm). In diabetic group GSH was significantly lower and LPO was significantly higher than control group. Glurenorm caused significant decrease in the GSH level of both control and diabetic aorta tissues and caused significant increase in the LPO levels of control aorta tissues and significant decrease of diabetic aorta tissues. Total protein levels of aortas were not found to differ significantly between all groups.

The protein bands obtained by Laemmli SDS-PAGE were in the same position for every sample and found at the same molecular weights (Data not shown).

in diabetes [14,15]. DM also alters arterial wall compliance and causes aortic stiffness and this may contribute to the increase in mortality and morbidity associated with DM [16-18]. As hyperglycemia activates protein kinase C, elevated glucose levels stimulate reactive oxygen species (ROS) production via protein kinase C (PKC)-dependent activation of NAD(P)H oxidase [19-21]. Glurenorm (gliquidone) is a one of the second generation sulphonyureas and is commonly used in the treatment of type 2 diabetes mellitus. In the present study, administration of glurenorm (gliquidone, 10 mg/kg) to rats inhibited diabetic blood glucose increase and body weight decrease, which is consistent with other studies [23-25]. It caused to decrease LPO levels in diabetic rat aortas but unfortunately it caused to increase NEG of aorta proteins and decrease GSH level of aorta tissue in diabetic rats. To our knowledge, however, there are no reports on neither oxidant nor antioxidant effects of glurenorm on aorta in diabetes. Glurenorm seems likely that has some oxidant effects on aorta as it increased LPO and decreased GSH levels of control group rats.

It was revealed that, despite their beneficial effects, continuous use of sulfonylureas may cause pancreatic β-cell dysfunction and apoptosis [26]. Sustained enhancement of Ca^{2+} influx by sulfonylureas may have been a causative mechanism for β-cell apoptotic cell death [27]. These studies are in accordance with our findings which shows the LPO increase and GSH decrease in the aortas of control rats

Table 1. *Effect of glurenorm treatment on body weight and blood glucose levels of STZ-induced diabetic rats*

Tablo 1. *STZ ile diyabet oluşturulmuş sıçanların vücut ağırlığı ve kan glukoz seviyeleri üzerine glurenorm tedavisinin etkisi*

Parameters	Control (1) (n=7)	Control+glurenorm (2) (n=15)	Diabetic (3) (n=8)	Diabetic+glurenorm (4) (n=13)	P ANOVA
Body weight (g)	206.01±13.05	169.27±10.99	144.93±21.16	152.04±28.06	0.0001
Blood glucose (mg/dL)	53.63±2.64	34.86±7.17	158.47±56.94	87.30±25.64	0.0001

Values are given as mean ± SD (SD: Standart deviation). Important significant differences, where [a] : P<0.01. For example, blood glucose (1- 3) [a] means that the difference in blood glucose between groups 1 and 3 is significant for P<0.01
Body weight (1-2, 1-3, 1-4, 2-3, 2-4, 3-4) [a], Blood glucose ((1-2, 1-3, 1-4, 2-3, 2-4, 3-4) [a]

Table 2. *Effect of glurenorm treatment on total protein, NEG, GSH and LPO levels of STZ-induced diabetic rats*

Tablo 2. *STZ ile diyabet oluşturulmuş sıçanların total protein, NEG ve GSH seviyeleri üzerine glurenorm tedavisinin etkisi*

Parameters	Control (1) (n=7)	Control+glurenorm (2) (n=15)	Diabetic (3) (n=8)	Diabetic+glurenorm (4) (n=13)	P ANOVA
Total protein (mg protein/g aorta)	24.64±3.19	24.71±4.09	26.36±2.41	25.16±4.73	0.770
NEG (nmol fructose/mg protein)	2.25±0.78	2.63±0.26	3.95±1.02	6.14±1.37	0.0001
GSH (mg GSH/mg protein)	14.27±4.07	3.46±0.69	8.60±1.97	1.53±0.53	0.0001
LPO (nmol MDA/mg protein)	0.11±0.04	0.41±0.05	0.98±0.32	0.49±0.12	0.0001

Values are given as mean ± SD (SD: Standart Deviation). Important significant differences, where [a] : P<0.01. For example, blood glucose (1- 3) [a] means that the difference in blood glucose between groups 1 and 3 is significant for P<0.01
NEG: Nonenzymatic glycation, GSH: Reduced glutathione, LPO: Lipid peroxidation, MDA: Malondialdehyde
NEG (1-3, 1-4, 2-3, 2-4, 3-4)[a] GSH (1-2, 1-3, 1-4, 2-3, 2-4, 3-4)[a] LPO (1-2, 1-3, 1-4, 2-3, 3-4)[a]

DISCUSSION

Vascular complications associated with diabetes are the major cause for the increased morbidity and mortality by glurenorm administration. Therefore, it is likely that in the present study glurenorm might have stimulated ROS production in aorta via the same mechanism as other sulfonylureas, but at the same time diminishes oxidative

stress via its radical scavenging effect. It was revealed that glurenorm has a protective effect on the damage caused by diabetes on liver tissue [28]. In our previous study, glurenorm was found to decrease oxidative stress in lenses but did not change the oxidant status seen in the skins of diabetic rats [29].

As diabetes is well known to cause endothelial dysfunction associated with an increase in oxidant stress [16,30], including when streptozotocin is used to induce diabetes in rats [31,32], any benefit of antioxidants on vascular function have to be demonstrated.

Glurenorm, in doses used, decreased LPO levels but did not reversed the NEG and GSH levels. Glurenorm administration also did not change the electrophoretic pattern of aorta tissue proteins. Therefore, to determine the overall efficacy of glurenorm for treating diabetes, sensitive tests of aorta may have to be conducted.

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