

Effects of Gliclazide on Uric Acid and C-reactive Protein in Alloxan Induced Diabetic Rats

L.S. Ojulari^{*1}, S.A. Biliaminu², E.O. Dangana¹, F.I. Abdulazeez³, T.O. Ayinde¹,
O.A. Adegoke⁴

¹Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.

²Department of Chemical Pathology and Immunology, University of Ilorin, Ilorin, Nigeria.

³Department of Pharmacy, University of Ilorin, Ilorin, Nigeria.

⁴Department Physiology, College of Medicine, University of Lagos, Nigeria (E-mail: feviade@hotmail.com)

(Received October 6, 2012; Accepted November 10, 2012)

ABSTRACT: **Background:** Diabetes mellitus represents one of the greatest threats to modern global health. It contributes to oxidative stress and also induces inflammation and hence severe complications. Several drugs have been introduced so far to salvage this metabolic disease alongside its complications. **Objectives:** This study was designed to investigate the effects of gliclazide on serum uric acid and C-reactive protein (a biomarker of inflammation) in alloxan-induced diabetic rats. **Materials and Methods:** Sixteen wistar rats were divided into 4 experimental groups with four rats each; Group A-control (Drug vehicle), Group B-diabetic, Group C- diabetic/gliclazide (10mg/kg twice daily for 28days) and Group D-normal/gliclazide (10mg/kg twice daily for 28 days). At the end of the experimental period (four weeks), animals in all groups were fasted for 12 hours and blood samples were taken by cardiac puncture for determination of serum uric acid and C-reactive protein (CRP) levels. **Results:** The study shows no significant statistical change in the serum uric acid levels ($p>0.05$) when the Experimental groups were compared with the controls. On the other hand, there was significant decrease ($p<0.05$) in CRP levels when values in the controls were compared with diabetic treated and normal treated groups. **Conclusion:** This finding may suggests that gliclazide possesses cardio-protective property since CRP has been implicated in atherosclerotic changes which is a common complication of diabetes mellitus. This may be through its anti-inflammatory effect by reducing the plasma concentration of IL-6, which is produced predominantly by macrophages and so prevents diabetic complications.

Keywords; Gliclazide, diabetes mellitus, uric acid, C-reactive protein

*Author to whom correspondence should be addressed.
E-mail: ojularis@yahoo.com, Phone: +2348032429067

Introduction

Diabetes mellitus, simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because the cells do not respond to the insulin that is produced. It represents one of the greatest threats to modern global health. In order to overcome the consequences, a group of drugs known as anti-diabetic drugs have proved to improve insulin sensitivity.

Some of the medications used so far in the management of the different classes of diabetes are the sulfonylureas which include tolbutamide, and chlorpropamide the “first generation sulfonylureas and the second generation sulfonylureas which include gliclazide. These have been introduced for clinical use since 1942 and they stimulate the release of insulin by binding to sulfonylurea receptors (SUR-1) on the surface of pancreatic beta cells with consequent closure of K^+/ATP , depolarisation and uptake of calcium into the cytoplasm. The rise in the intracellular calcium leads to increased insulin secretion. They are variously metabolised in the liver and excreted in the bile and urine¹.

Gliclazide is an oral hypoglycemic antidiabetic drug and classified as a sulfonylureas². It has been proved to protect human pancreatic beta cells from antiatherogenic effect in type 2 diabetes³. Gliclazide is used for control of hyperglycaemia in gliclazide-responsive diabetes mellitus of stable, mild, non-ketosis prone, type 2 diabetes. It is used when insulin cannot be controlled by proper management and exercise or when insulin therapy is not appropriate. Its characteristic properties are: restoring first peak of insulin secretion; increasing insulin sensitivity; glycaemic-dependent haemovascular effects; anti-oxidant effect; no active circulating metabolites³. The main goal of antidiabetic therapy, as already noted, is to improve the diabetic condition. They, as already noted, are to prevent some diabetic complications. There are views on changes of serum acute phase protein levels and reports suggesting the relationship between gliclazide and inflammation in diabetes⁴. In this context, it has been observed that as the anti-diabetic therapy plays its major role, it also has effect on other parameters of the body such as uric acid, platelets, and haemoglobin.

C-reactive protein (CRP) is a protein found in the blood. It is produced in the liver and the level rises in response to inflammation. It is an acute phase protein and its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells and some types of bacteria in order to activate the complement system V1a, the C1Q complex. The normal concentration in healthy human serum is usually lower than 10mg/l, slightly increasing with age. High levels are found in late pregnant woman, mild inflammation and viral infections (40-200mg/l), severe bacterial infections and burns (>200mg/l)⁵. Research suggests that patients with elevated basal levels of CRP are at risk of diabetes^{6,7}. CRP rises to about 50,000 folds in acute inflammation such as infection. It rises above normal limits within 6hrs and peaks at 48hrs. Its half-life is constant and, therefore, its level is mainly determined by rate of production⁵.

Uric acid is a heterocyclic compound which is created when the body breaks down purine nucleotides. It has a low water solubility which is significant for the etiology of gout, a condition of high concentrations of uric acid in blood serum. Uric acid is released

in hypoxic conditions and is the final oxidation product of purine metabolism; it is also excreted in the urine.

In humans, over half of the anti-oxidant capacity of blood plasma comes from uric acid and the reference range in blood plasma is between 3.6mg/dl and 8.3mg/dl. Hyperuricaemia is associated with several risk factors for cardiovascular diseases such as hypertension, insulin resistance, and diabetes⁸. Serum uric acid is slightly reduced in type 2 diabetic patients especially when it is complicated and this may result from the oxidative stress that decreases the anti-oxidant capacity of the body involving uric acid. Gliclazide reduces oxidative stress in type 2 diabetic patients by improving plasma anti-oxidant status, and this effect is associated with enhanced nitric oxide-mediated vasodilation⁹. (Fava et al., 2010).

With these relationships between diabetes mellitus and C-reactive protein or uric acid, this study therefore, seeks to further evaluate the anti-inflammatory/anti-oxidant effects of gliclazide by its effect on serum uric acid and C-reactive protein in alloxan induced female diabetic rats.

Materials and Methods

Experimental Protocol

Sixteen female albino wistar rats (weighing between 130g to 150g) were maintained under standard laboratory conditions and were allowed free access to food and water. Animals were divided into four experimental groups. Group A- Control; Group B- Diabetic-untreated; Group C- Diabetic/gliclazide (10mg/kg twice daily for 28 days); and Group D- Normal/gliclazide (10mg/kg twice daily for 28 days).

Induction of Diabetes

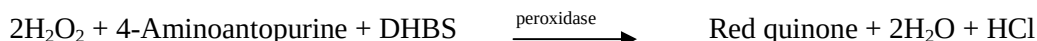
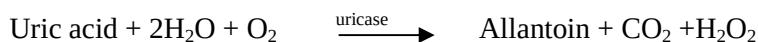
Diabetes was induced by a single intraperitoneal injection of 100mg/kg of alloxan monohydrate obtained from Sigma Chemical Co, (St. Louis, MO, USA). Diabetes was confirmed by glucose oxidase method using glucometer (One Basic, Inc.). After 72 h of alloxan injection, rats with plasma glucose level ≥ 200 mg/dl were separated and used as diabetic in this study.

Drugs, Route and Duration of treatment

The drug gliclazide 80mg was dissolved in 20ml of distilled water before it was administered. Dose selected was 10mg/kg¹⁰. The suspension was administered orally with oral cannula, twice daily, for 28 days. At the end of the experimental period, rats were fasted for 12 h and sacrificed. Blood was collected by cardiac puncture and transferred into plain bottles for biochemical analysis.

Biochemical Analysis:

The evaluation of uric acid using uricase method which is the most feasible and popular as a result of the availability of high quality, low-cost preparations of the bacterial enzyme was carried out according to the following reactions and the reaction observed in kinetic mode based on the absorbance using a spectrophotometer.



DHBS= 3,5- Dichloro-2- Hydroxybenzene sulphonic acid

Uricase acts as oxidoreductase

Peroxidase acts as oxygen acceptor to yield a chromogen in the visible spectrum.

The reagents were mixed and incubated for 5mins at 37°C after which the absorbance of sample and standard solution against reagent blank was measured.

Calculation

$$\text{Uric acid conc. (mg/dl)} = \frac{\text{Absorbance of sample} \times \text{conc. of standard solution}}{\text{Absorbance of standard}}$$

The biochemical analysis of CRP was done using high sensitive CRP turbidatex agglutination kit manufactured by Agappe (Switzerland) and supplied by NUMS diagnostic Nigeria Ltd. Latex principles coated with specific human anti-CRP in agglutination causes absorbance change depending upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known concentration.

Calculation

CRP conc. (mg/l) = $A_2 - A_1$ (sample) / $A_2 - A_1$ (calibrator) multiplied by the calibrator's concentration.

Biochemical analysis of serum glucose was done using Glucose Kit manufactured by Agappe (Switzerland) and supplied by NUMS diagnostic Nigeria Ltd. The glucose analysis was based on glucose oxidase principle.

Statistical Analysis:

All results were expressed as mean $\pm$ SEM. Data was analysed by one-way analysis of variance (ANOVA) and Duncan New Multiple Range Test (DMRT). Differences in means were considered significant at $P < 0.05$. All analyses were performed using SPSS Version 17.

Results

Mean values of serum uric acid and C-reactive protein of control and Experimental groups are shown in Table 1. Mean and standard error of mean (S.E.M) of uric acid level in Group A-control group (drug vehicle) was 23.05 ± 14.59 mg/dl, Group B-diabetic

9.30±0.14mg/dl, Group C-diabetic/gliclazide (10mg/kg twice daily for 28 days) 8.65±0.16mg/dl and Group D-normal/gliclazide (10mg/kg twice daily for 28 days) 23.21±10.95mg/dl. There was no significant statistical change in the serum uric acid level ($p>0.05$) when Experimental groups' result was compared with that of the control group.

Table 1: Effect of gliclazide on serum uric acid and C-reactive protein in control and experimental rats.

Variables/ Groups	A Control (drug vehicle) (n=4)	B Diabetic (n=4)	C Diabetic/Gliclazide (10mg/kg) (n=4)	D Normal/gliclazide (n=4)
Serum Uric acid(mg/dl)	23.05±14.59	9.30±0.14	8.65±0.16	23.21±10.95
Serum CRP(mg/l)	36.90±8.98	87.73±49.05	17.60±0.00 ^b	1.05±6.75 ^a
Serum glucose(mg/dl)	139.75±6.20	277.75±38.58 ^a	162.75±5.97 ^b	134.85±5.11

Values are expressed as mean ±S.E.M, ^a $p<0.05$ when compared to control, ^b $p<0.05$ when compared to group B (Diabetic). CRP=C-reactive protein. n=number of rats.

The mean and S.E.M of serum CRP in Group A-control group (drug vehicle) was 36.90±8.98mg/l, Group B- diabetic 87.73±49.05mg/l, Group C-diabetic/gliclazide (10mg/kg twice daily for 28 days) 17.60±0.00mg/l and Group D-normal/gliclazide (10mg/kg twice daily for 28 days) was 1.05±6.75mg/l. There was significant decrease ($p<0.05$) when control was compared with Group C (diabetic/gliclazide) and Group D (normal/gliclazide) while there was no significant statistical difference ($p>0.05$) when compared with that of Group B (diabetic).

Discussion

Recently, focus has been on the role of oxidative stress in the development of diabetic complications and the major source of oxidative stress in diabetes has been observed to be reactive oxygen species (ROS) induced by high glucose directly damaging cells via oxidation of cell membrane. Gliclazide has been recommended for use in its glucose lowering effects in diabetes. It also has beneficial effects on improving free radical status through its antioxidant properties¹⁰. Uric acid is a variable in the body which accounts for about half of the antioxidant capacity of the body system. Studies also show that

gliclazide significantly reduce high glucose induced apoptosis as a result of its anti-oxidant properties¹¹.

The association of high serum uric acid with insulin resistance has been known since the early part of the 20th century. Nevertheless, recognition of high serum uric acid as a risk factor for diabetes has been a matter of debate. In fact, hyperuricaemia has always been presumed to be a consequence of insulin resistance rather than its precursor¹². However, a prospective follow-up study showed that high serum uric acid is associated with higher risk of type 2 diabetes, independent of obesity, dyslipidaemia, and hypertension⁷. Some researchers propose that hyperuricaemia-induced oxidative stress is a cause of metabolic syndrome^{13,14}.

Our results showed that gliclazide does not significantly ($p>0.05$) affect the uric acid level when administered orally and at doses and duration administered in diabetic rats. This is in contrast to the study by Fava et al⁹, who observed a slight reduction in uric acid level in type 2 diabetic patients, particularly in the complicated patients with peripheral nephropathy and oxidative stress, on treatment with gliclazide. Also, in his observation, Paul (a Consultant Physician in Diabetes and Endocrinology in New York), noticed that in diabetes, where increased glycation and oxidation are fundamental to the pathogenesis of diabetic vascular disease, agents such as gliclazide with its antioxidant activities may have an enhanced therapeutic role¹⁵. The difference in these observations may be due to duration of administration of the agent gliclazide, subjects used (in this research work) as well as environmental conditions.

CRP is a member of the class of acute phase reactants, (precisely positive acute phase protein) as its level rises dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages⁵ as well as adipocytes¹⁶.

Research suggests that patients with elevated basal levels of CRP are at an increased risk of diabetes, hypertension and cardiovascular disease^{6,7}. Others have shown that CRP can exacerbate ischemia necrosis in a compliment-dependent fashion and that CRP inhibition can be a safe and effective therapy for myocardial and cerebral infarcts. So far, this has been demonstrated in animal model. There is a strong association between baseline CRP concentration and BMI; and weight loss lowers the CRP values. Raised base-line CRP values are also associated with many features of the insulin resistance or metabolic syndrome¹⁷ up to and including frank diabetes mellitus¹⁸. Indeed, CRP production predicts the development of type 2 diabetes mellitus independently of traditional risk factors¹⁸. In insulin-resistant obese individuals, elevated CRP values fall in parallel with improvements in insulin resistance.

From the result of our research, it was observed that there was a significant decrease ($p<0.05$) in the level of CRP following a 28 day administration of gliclazide, orally, in normal and diabetic rats. Gliclazide reduces the level of CRP production probably by reducing the level of plasma concentration of IL-6⁵. Our findings correspond to that by Pepys et al⁵, in which gliclazide decreased the rate at which CRP was produced in diabetes mellitus and which could in turn decrease the development of certain complications. Also, Collier et al. observed that gliclazide decreases the development of diabetic complications and inflammation by decreasing CRP production⁴.

From our research, it could be deduced that gliclazide probably has no significant effect on uric acid level but reduces CRP production in diabetes mellitus through its anti-inflammatory effect by reducing the plasma concentration of IL-6 (produced predominantly by macrophages) and so prevents diabetic complications. It is known that there is a relationship between diabetic complications and chronic inflammation characterized by alterations in circulating acute phase proteins (CRP). It has also been emphasized that inflammation contributes to diabetic complications and that gliclazide decreases the development of such complications¹⁹. Hence, in diabetes mellitus treated with this sulfonylurea (gliclazide), inflammatory diseases may be reversed as well as prevented by acting on this acute phase protein.

References

- (1) Young, J and Anwar: "Metabolism of sulphonylureas" *Current Diabetes Reviews* 5:252-258. 2009
- (2) Sultanpur, S, Satyanarayana, and Deepa K, "characteristic properties of gliclazide in animal model" *pharmacologia*, 2:201-205. 2011
- (3) Guerra, Del, S, Grupillo, M, Masini, M, Lupi, R, Bugliani, M, Torri, S, Boggi, U, Chiaro, M, Vistoli, F, Mosca, F, Pratto, S and Marchetti, P: "Gliclazide antiatherogenic effect in type 2 diabetes". 2006
- (4) Collier, A, Watson, HH, Patrick, AW, Ludlam, CA and Clarke, BF: *Diabetes Metabolism* 15(6):420-5. 1989
- (5) Pepys, MB and Hirschfield, GM: C-reactive protein: *a critical update* 12:1805-12. 2003
- (6) Pradhan AD, Manson JE, Rifai N, Buring JE & Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jour of the Amer Med Associa* 286 327–334. 2001
- (7) Dehghan, A, Kardys, I, Maat, MP, Uitterlinden, AG, Sijbrands, EJ, Bootsma, AH, Stijnen, T and Hofman: "Genetic variation, C-reactive protein levels, and incidence of diabetes". *Diabetes jouunal* 56 (3). 872-8. 2007
- (8) Perticone, F, Sciacqua, A, Perticone, M, Arturi, F, Scarpino, PE, Quero, M and Sesti, G: "Hyperuricemia associated with several risk factors for cardiovascular disease and diabetes" *Diabetic care journal pharmacologic* 2:201-205. 2011
- (9) Fava, D, Cassone-Faldetta, M, Laurenti, O, Luca, O, Ghiselli, and Mattia, G: "Gliclazide reduces oxidative stress in type 2 diabetes" *Diabetic Med.* 27 (9):1041-8. 2010
- (10) Alberti, KG, Johnson, AB and Taylor, R: "Beneficial effects of gliclazide on improving free radical status through its antioxidant properties" *Human Diabetes and Metabolic Research Centre*, University of Newcastle. 2007
- (11) Del Rio D, Brighenti F, Valtueña S, Pellegrini N, Ardigo D: Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. *Br J Nutr* 93: 619-625. 2005
- (12) Cappuccio FP, Strazzullo P, Farinaro E: Uric acid metabolism and tubular sodium handling. *JAMA*; 270:354. 1993
- (13) Nakagawa, T, Hu, H and Zharikov, S: "A causal role for uric acid in fructose-induced metabolic syndrome". *American Journal of Physiology. Renal Physiology* 290 (3): F625–31. 2006

- (14) Hayden, MR and Tyagi, SC: "Uric acid: A new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: *The urate redox shuttle*". *Nutrition & Metabolism* 1 (1): 10. 2004
- (15) Paul, E: "increase glycation and oxidation are fundermental to the pathogenesis of diabetic vascular disease" *Jennings Volume 49, Issue 10, Supplement 2*, Pages 17–20. 2000
- (16) Lau, DC, Dhillon, B, Yan, H, Szmitko, PE and Verma, S: "CRP increment due to rise in IL-6 produced predominantly by adipocytes *Am J Physiol Heart Circ Physiol* **288** (5): H2031–41. 2005
- (17) Jonathan, E: "C-reactive protein concentration and the vascular benefits of statin therapy: *an analysis of 20,536 patients in the Heart Protection Study*". *Lancet* 377 (9764): 469–76. 2011
- (18) Deron, SJ: C-reactive protein. *American heart Association* TX75231 (800) 242-8721. 2003
- (19) Ceriello, A: Effects of gliclazide beyond metabolic control metabolism. *Metabolism* 55(5 suppl 1):10-15, 2006