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Blood Glucose Level, Pancreatic Histology and Insulin-Expression in Diabetic Rats Following Metformin and Glibenclamide Administration

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Abstract

Metformin and Glibenclamide are used in the management of type 2 diabetes mellitus (T2DM). There is paucity of information on the efficacy of insulin expression by these anti-diabetic drugs. This study investigated the hypoglycemic, pancreatic histomorphological and insulin alterations following administration of Metformin and Glibenclamide in a T2DM model. Thirty Wistar rats were divided into six groups of five animals each. Two groups served as normal and diabetic controls respectively. Diabetes was induced with Streptozotocin (STZ). Two diabetic groups received 1.43 and 2.86 mg kg^{-1} body weight (bw) Metformin, while another two groups received 0.07 and 0.14 mg kg^{-1} bw Glibenclamide respectively. Treatments lasted for four weeks, after which animals were fasted over-night before determination of final blood glucose levels. Pancreas was dissected for histological study. Hypoglycemic effect of Glibenclamide was higher than that of Metformin. The histological features of Glibenclamide-treated rats demonstrated severe distortions, while Metformin-treated rats had mild distortions of the pancreatic islets. Insulin expression was strongly enhanced by Metformin than by Glibenclamide. In conclusion the oral therapeutic doses of Glibenclamide had a higher hypoglycemic action than Metformin. Metformin however had a stronger attenuation of diabetes-induced pancreatic distortions, and up-regulates insulin expression in diabetic rats than Glibenclimide.

Keywords : *Diabetes, Glibenclamide, Metformin, blood glucose, pancreas, insulin immunohistochemistry.*

Introduction

Diabetes mellitus is a serious chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Diabetes is an important public health condition and one of the four priority non-communicable diseases (NCDs) targeted for action by world leaders. The global estimates of diabetes have risen from 108 million in 1980 to 422 million as at 2014 [1], a prevalence rising more rapidly in low and middle-income countries, attributable to the adoption of western life style especially in diet.

A first line medication for the treatment of type 2 diabetes, particularly in people who are overweight is Metformin, marketed as Glucophage® [2]. Metformin decreases blood glucose levels by decreasing hepatic glucose production (gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral uptake and utilization of glucose [3]. The drug has cardiovascular protective effects independent of glucose-lowering effect [4], and is sometimes co-administered with Glibenclamide.

Glibenclamide (also known as Glyburide) is an anti-diabetic drug in a class of medications known as Sulfonylureas, closely related to sulfonamide antibiotics. It is the most popular sulfonylurea used in the treatment of type 2 diabetes mellitus (T2DM) in the

United States [5]. It is not as good as either Metformin or insulin in those who have gestational diabetes [6]. The drug has been reported to be a major cause of drug-induced hypoglycemia [7], and may cause acute haemolysis, if administered to persons having glucose -6- phosphate dehydrogenase deficiency [8]. Glibenclamide works by binding the ATP-sensitive potassium channels in pancreatic β-cells, which results in an increase in intracellular calcium in the β-cells of the pancreas and subsequent stimulation of insulin release [9].

Diabetes can be induced in rodents by Streptozotocin acting by inhibition of β-cell O-linked N-acetylglucosamyl hydrolase [10] and donation of nitric oxide (NO), involved in many physiological and pathological processes in the body [11]. Beta cells are clusters of cells in the pancreatic islets of Langerhans that secrete insulin, the anti-hyperglycemic hormone. This study investigated the effects of Metformin and Glibenclamide on blood glucose levels, pancreatic histology and insulin immunohistochemical expression in experimental diabetic model.

Materials and Methods

Experimental Animals

Thirty adult male Wistar rats were obtained from the Faculty of Basic Medical Sciences Animal House and acclimatized for two weeks before use, under optimum environmental conditions of temperature 25 ± 5 ^{o}C with 12 hours light and dark cycle. The animals were allotted into well–maintained plastic cages. The animals were fed with pelletized growers mash (Grand Cereal Vital® Feed Ltd., Jos, Nigeria) and given tap water *ad libitum*. Ethical approval was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences of University of Uyo, Nigeria. The ethics of animal care were adopted following the "Guide for the Care and Use of Laboratory Animals" [12].

Drug Acquisition

Streptozotocin (300 mg) was obtained from Santa Cruz Biotechnology, Inc., U.S.A. Metformin (Glucophage) was obtained from Merck S. L. Poligono Merck Ltd., Barcelona, Spain. Glibenclamide was obtained from Nigeria German Chemical Plc, Otta, Ogun State, Nigeria. Citrate buffer was obtained from Nanjing Shuguang Silane Chemical Co., Ltd., 5611EH Eindhoven, Netherlands. Normal saline and distilled water were obtained from the laboratory of the Department of Biochemistry, University of Uyo, Nigeria.

Induction of Type 2 Diabetes Mellitus

Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of freshly prepared Streptozotocin (STZ) at 50 mg/kg body weight (bw) dissolved in 0.1M citrate buffer (pH 4.5), as earlier described Lenzen [13]. Control animals were injected i.p with citrate buffer alone at a single dose of 1.2 mL/kg bw. Diabetes was confirmed three days after STZ administration. Thereafter, fasting blood glucose levels of the animals were measured using a Glucometer (ACCU-CHECK Advantage II, Roche Diagnostics, GmbH, Germany). Following the criteria of previous workers, rats with blood glucose levels of more than 198 mg/dL were considered to be diabetic and therefore used for the study [14 - 15].

Experimental Design

The thirty rats were allotted into six groups of five rats each. Group 1 served as the normal control (NC), which received 0.5 mL normal saline. Groups 2 to 6 received intraperitoneal injection of STZ (50 mg per kg bw) of animal. Group 2 served as the diabetic control (DC), which did not receive any drug treatment. Groups 3 and 4 (D_{LM} and D_{HM}) received 1.43 and 2.86 mg/kg Metformin, while Groups 5 and 6 (D_{LG} and D_{HG}) received 0.07 and 0.14 mg/kg Glibenclamide respectively. Additionally 0.5 mL normal saline was administered to animals in all test groups. The duration of the experiment was twenty-eight days.

Determination of Blood Glucose

Blood glucose concentration was estimated by glucose oxidase method [16], using a reagent kit from Randox Laboratory Ltd, UK.

Histological Studies

Excised pancreas tissues were fixed in 10% neutral buffered formalin (NBF). After 24 hours, the tissues were processed following standard protocol [17], and embedded in paraffin wax. Pancreatic transverse sections of 5 µm thickness were cut with the rotary microtome (Microtome Thermo Scientific – Microm HM 325, England), and used for Hematoxylin and Eosin staining. Insulin antibody expression was evaluated with the scoring system reported by Klien *et al.* [18]. Photomicrographs of all slides were obtained under

light microscope (Olympus - CX31, Japan). Images were obtained with Amscope digital camera (MU 1000, China) attached to the microscope, and were blindly assessed by three independent histopathologists.

Statistical Analysis

Statistical analyses were performed using the Primer of Statistics software version 3.01. Data in this study were expressed as means ± standard error of mean (SEM) and analyzed using one-way analysis of variance (ANOVA) to determine the difference between the test groups compared to control, and the post-hoc test (Student-Newman Keuls) for between groups comparison. Values were regarded as statistically significant at $p < 0.05$.

Results

Effect of Metformin and Glibenclamide on Body Weights

There was a marked percentage gain in body weight of animals (g) in NC (+19.47) when compared with DC (+ 0.55), D_{LM} (+ 8.15), D_{HM} (+ 13.73), D_{LG} (- 2.7) and D_{HG} (+ 13.7), as shown in Table 1.

Effect of Metformin and Glibenclamide on Blood Glucose Levels

The STZ-induced diabetes was evident from blood glucose readings of the day, obtained 72 hours post-induction after overnight fasting of the rats (Figure 1), showing significant increase in blood glucose in the test groups compared to NC. However, consequent administration of Metformin and Glibenclamide to STZ-treated groups showed trends of blood glucose attenuation, though rats were still considered diabetic. Overall, Glibenclamide elicited more hypoglycemic effect than Metformin in this study.

Effect of Metformin and Glibenclamide on Pancreatic Histology and Insulin expression

Histologically, NC group demonstrated well stained pancreatic islets (Pi) with good nuclei morphology and cytoplasm (Fig 2 [NC]). The DC rats had hypertrophied Pi nuclei, atrophied Pi cytoplasm and few necrotic nuclei of Pi with vacuolations (Fig 2 [DC]). The DM groups presented few hypertrophied, atrophied and polymorphic nuclei of Pi, with prominent vacuolations. The DM groups exhibited numerous Pi nuclei and decreased Pi cytoplasmic volume (Fig 2 [D_{LM} & D_{HM}]). The rats in the group D_{LG} indicated fatty necrosis especially on serous acini with vacuolations, while D_{HG} rats had hypertrophied Pi nuclei (Fig 2 [D_{LG} & D_{HG}]).

Immunohistochemically, the insulin antibody expression revealed (as shown in Fig 3) that there was high insulin expression in the NC, D_{LM} and D_{LG} groups compared with low insulin expression in the DC, D_{HM} and D_{HG} groups. The corresponding immunohistochemical scores are presented in Table3.

Table 1. *Effect of Metformin and Glibenclamide Administration on Total Body Weights**

Body Weight (g)	Experimental Group					
	NC	DC	D_{LM}	D_{HM}	D_{LG}	D_{HG}
Initial	127.00 ± 5.81	178.20 ± 5.84	160.40 ± 3.89	142.00 ± 0.63	157.00 ± 0.84	145.60 ± 5.88
Final	160.20 ± 3.99	179.00 ± 4.68	174.20 ± 5.54	164.60 ± 4.49	152.80 ± 11.21	168.80 ± 16.70
Weight change (%)	+19.47	+0.55	+8.15	+13.73	-2.7	+13.7

*Data are expressed as Means ± SEM (n = 5 rats per group).

Legend: NC = Normal Control

DC = Diabetic Control

D_{LM} = Diabetic rats treated with low dose metformin1.43 mg/kg body weight (bw)

D_{HM} = Diabetic rats treated with high dose metformin 2.86 mg/kg bw

D_{LG} = Diabetic rats treated with low dose glibenclamide 0.07 mg/kg bw

D_{HG} = Diabetic rats treated with high dose glibenclamide 0.14 mg/kg bw

Table 2. *Effect of Metformin and Glibenclamide Administration on Insulin Expression***

Group	% of IHC	(B) Intensity of IHC	Final score (A+B) for Insulin Antibody Expression*	Degree of Insulin Antibody Expression
NC	> 60 (3)	Strong (3)	6	High
DC	< 30 (1)	Mild (2)	3	Low
D_{LM}	> 60 (3)	Strong (3)	6	High
D_{HM}	< 30 (1)	Mild (2)	3	Low
D_{LG}	< 30 (1)	Mild (2)	3	Low
D_{HG}	> 60 (3)	Strong (3)	6	High

** Values in parenthesis indicate score for % and intensity of IHC.

Legend: IHC = Immunohistochemistry; NC = Normal Control; DC = Diabetic Control; D_{LM} = Diabetic rats treated with low dose metformin 1.43 mg/kg body weight (bw); D_{HM} = Diabetic rats treated with high dose metformin 2.86 mg/kg bw**;** D_{LG} **=** Diabetic rats treated with low dose glibenclamide 0.07 mg/kg bw**;** D_{HG} = Diabetic rats treated with high dose glibenclamide 0.14 mg/kg bw; **Key:** % IHC: 0 = 0%, 1 = < 30%, 2 = 30 – 60 %, 3 = < 60 %; Intensity of IHC: 0 = No reaction, 1 = Weak, 2 = Mild, 3 = Strong; * Final Score: A+B (Range from 0 to 6): 0/6= Negative Reaction; 1/6 to3/6 = Low expression; 4/6 to 6/6 = High expression.

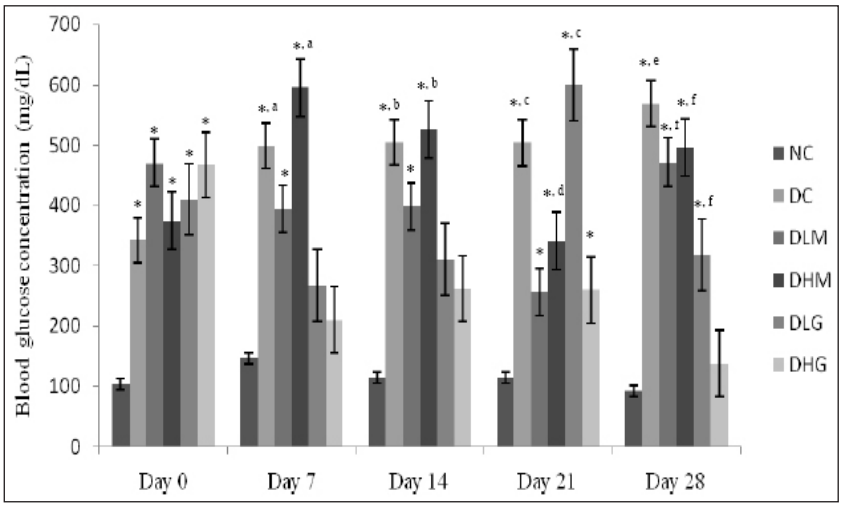

Figure 1. *Effect of Metformin and Glibenclamide Administration on Blood Glucose Levels*

Data are expressed as Means ±SEM; (n = 5 rats per group).

*** = Significantly increased compared to NC ($p < 0.05$)

[a] = Significantly increased compared to D_{LG} and D_{HG} ($p < 0.05$)

[b] = Significantly increased compared to D_{HG} ($p < 0.05$)

[c] = Significantly increased compared to $D_{LM,}$ $D_{HM,}$ and D_{HG} ($p < 0.05$)

[d] = Significantly increased compared to D_{LM} ($p < 0.05$)

[e] = Significantly increased compared to D_{LG} and D_{HG} ($p < 0.05$)

[f] = Significantly increased compared to NC and D_{HG} ($p < 0.05$)

Legend: NC = Normal Control; DC = Diabetic Control; D_{LM} = Diabetic rats treated with low dose Metformin 1.43 mg/kg body weight (bw); D_{HM} = Diabetic rats treated with high dose Metformin 2.86 mg/kg bw; D_{LG} **=** Diabetic rats treated with low dose Glibenclamide 0.07 mg/kg bw**;** D_{HG} = Diabetic rats treated with high dose Glibenclamide 0.14 mg/kg bw

Histopathological Assessment

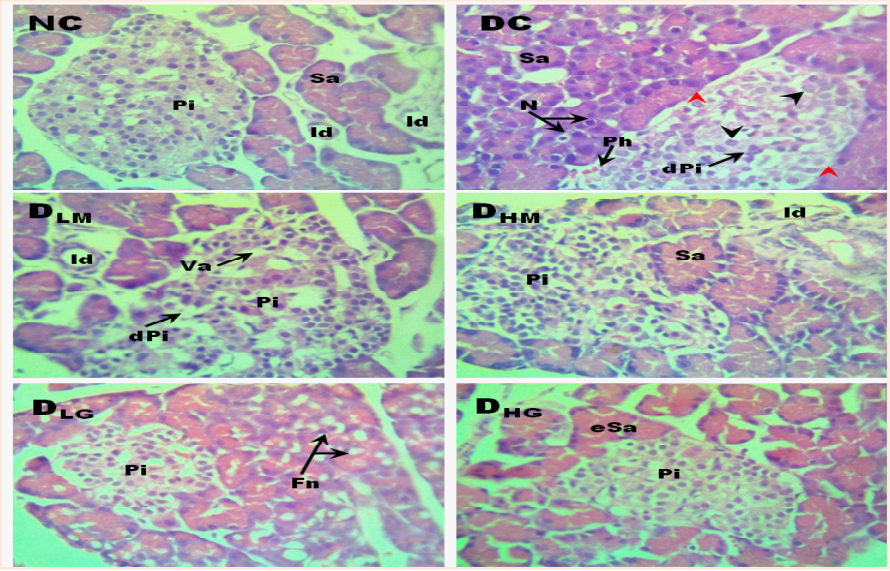

Figure 2. *Photomicrographs of transverse section of the pancreas. (H&E) x400*

NC = Normal control (appears normal); DC = Diabetic control (severely affected)

D_{LM} = Diabetic rat treated with low Metformin 1.43 mg/kg body wt (bw) (moderately affected)

D_{HM} = Diabetic rat treated with high dose Metformin 2.86 mg/kg bw (moderately affected)

D_{LG} = Diabetic rat treated with low dose Glibenclamide 0.07 mg/kg bw (severely affected)

D_{HG} = Diabetic rat treated with high dose Glibenclamide 0.14 mg/kg bw (moderately affected)

Key: Red arrow head = oedema, black arrow head = hypertrophied nuclei, N = necrosis; Sa = Serous acini; eSa = Esinophilic Serous acini; Pi = Pancreatic islets; dPi= degenerating and distorted Pancreatic islets; Fn = Region of fat necrosis; Id = Intralobular duct; Va = Vacuolation

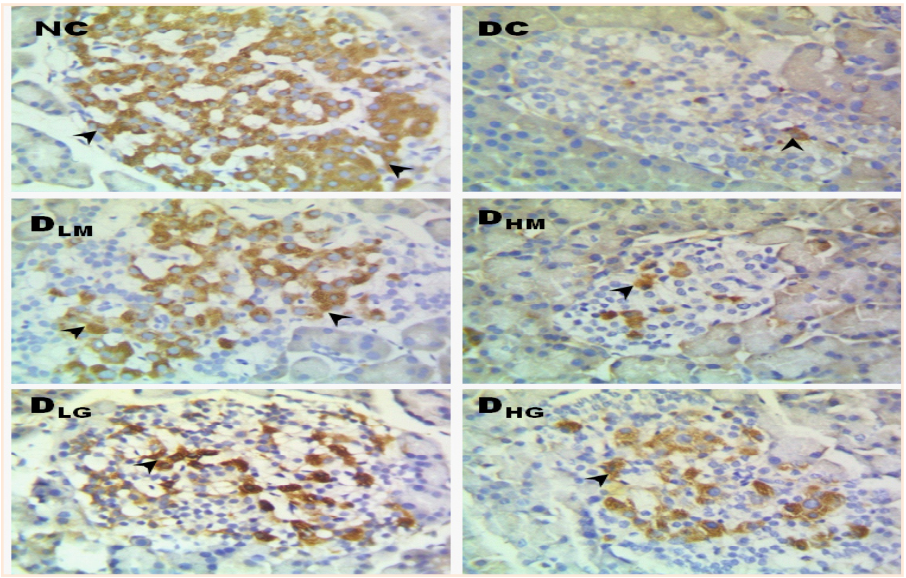

Figure 3. *Photomicrographs of transverse section showing insulin antibody expression (black arrow head). Strongly expressed in NC, D_{LM}, D_{LG} and D_{HG}. Weakly expressed in DC and moderately expressed in D_{HM} (at ×400).*

Discussion

Well-known anti-diabetic drugs Metformin and Glibenclamide were investigated to compare their effects on blood glucose levels, pancreatic micro-anatomical alterations and intensity of insulin expressed by the pancreatic β-cells, as detected with insulin antibody marker in STZ diabetic rat model.

The blood glucose levels of the STZ - induced diabetic-treated groups were statistically reduced compared to the DC group. This result indicates that Metformin and Glibenclamide have anti-diabetic potentials as earlier documented [19]. This is consistent with the report of Okonkwo and Okoye [20], of a reduction in blood glucose levels in diabetic rats treated with Metformin. The blood glucose lowering effect of Metformin is dependent on the presence of insulin [21]. This correlates with the histological result of DC group, which demonstrated that administering STZ to rats did not completely degenerate pancreatic β-cells (solely responsible for insulin production), as insulin was expressed albeit in low intensity (Fig. 3 [DC]).

Histologically, the STZ-induced diabetic group revealed features of acute pancreatitis (Fig. 2.). Morphologic alterations of acute pancreatitis span from inflammation and edema to marked necrosis and hemorrhage. These morphologic alterations may show as: (i) microvascular leakage causing edema, (ii) necrosis of fat by lipolytic enzymes and (iii) an acute inflammatory reaction. The pancreatic alterations demonstrated in this study were ameliorated in the drug-treated groups, however not to the same extent.

Metformin promotes insulin binding to insulin receptors leading to reduction in blood glucose levels, suggesting that pancreatic β-cells are not completely destroyed in T2DM. Metformin has been reported to have antioxidant activity against oxidative damage caused by reactive oxygen species (ROS) [22]. The evidence of the attenuation of STZ-induced β-cell toxicity following Metformin administration is demonstrated in the intensity of insulin antibody expressed (Fig. 3 [D_{LM} & D_{HM}]).

Glibenclamide showed strong hypoglycemic potential. Insulin expression was strongly demonstrated in NC, D_{LM}, D_{HM} and D_{HG} groups. However, there were severe pancreatic islet distortions (acute pancreatitis) and high insulin expression in the Glibenclamide-treated group (Fig. 3 [D_{HG}]), when compared with Metformin group (Fig. 3 [D_{HM}]).

Glibenclamide reportedly stabilizes blood glucose by binding to and inhibiting the ATP-sensitive potassium channels through the inhibitory regulatory subunit, sulfonylurea receptor 1 (SUR1) in pancreatic β-cells. The inhibition causes cell membrane depolarization, opening of voltage-dependent calcium channel which increases intracellular calcium in the β-cells, and subsequent stimulation of insulin release [9].

Conclusion

The present study has shown that hypoglycemic effect of Glibenclamide was higher than that of Metformin. The histological features of Glibenclamide-treated rats demonstrated severe distortions, while Metformin-treated rats had mild distortions of the pancreatic microanatomy (islets of Langerhans). Insulin expression was strongly enhanced by Glibenclamide than by Metformin. The mechanism of action of Metformin (as regards insulin utilization) is presumed to be direct and a less energy-dependent pathway, whereas the inverse is the case for Glibenclamide. Thus the findings of this study suggest the use of Metformin as a better alternative to Glibenclamide in the amelioration of hyperglycemia, especially in the long term.

Conflict of Interest

Authors declare that there is none

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