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Research Article

Effect of Metformin on Potassium-adapted and Non-adapted Diabetic Rats

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Abstract

Purpose: *To assess the effects of potassium adaptation on some biochemical parameters in diabetic rats treated with metformin.*
Methods*: Diabetes was induced via intraperitoneal administration of streptozotocin in potassium-adapted and non-adapted rats and, then metformin (350 mg/kg) was administered orally. The following parameters were then evaluated 24 h after drug administration: urine volume, plasma glucose, plasma and urine creatinine and creatinine clearance. Lipid profiles and plasma and urine urea were also determined.*
Results: *The blood glucose of the potassium-adapted diabetic group was not significantly reduced on treatment with metformin, compared to non-adapted diabetic rats which were significantly reduced ($p < 0.05$) on metformin treatment. The potassium-adapted group treated with metformin had significantly higher creatinine clearance ($p < 0.05$) than the non adapted group. Urine volumes for both the diabetic and the potassium-adapted diabetic rats were significantly lower ($p < 0.05$) on treatment with metformin.*
Conclusion: *The results indicate that potassium adaptation induces resistance to treatment with metformin and increases creatinine clearance in diabetic rats.*

Keywords: *Metformin, hypoglycemia, potassium, adapted rats, non-adapted rats.*

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia as a result of the diminished production of insulin or mounting resistance to its action, hence tissues are unable to carry out the normal metabolism of carbohydrates, fats and protein. Chronic hyperglycemia during diabetes causes glycation of body protein thereby affecting eyes, kidney, nerves and arteries [1]. The classical symptoms of DM are polyuria (frequent urination), polydiapsia (increased thirst) and polyphagia (increased hunger) [2].

Metformin is used in non-insulin dependent diabetics without a tendency to ketosis in whom dietary carbohydrate restriction has not controlled hyperglycemia and who remain symptomatic. Its main use is in obese patients, because of its anorectic effect which aids weight reduction. The drug also exerts a useful additive effect in patients unresponsive to sulphonylureas alone. It is contra-indicated in patients at risk of lactic acidosis and in the following conditions; renal failure, alcoholics, cirrhosis, chronic lung disease, cardiac failure, mitochondrial myopathy, acute myocardial infarction and other serious intercurrent illness [3].

The role of potassium adaptation in the reduction of blood pressure has previously been demonstrated [4]. However, data on the effect of potassium adaptation on diabetes and in diabetic patients being treated with metformin are unknown. Hence, this study is geared towards providing information on the role of potassium adaptation in diabetic patients treated with metformin.

EXPERIMENTAL

Drugs and chemicals

Potassium chloride (Wells Brand, Nigeria), total cholesterol kit (Randox UK), triglyceride kit (Randox UK), high density lipoprotein kit (Randox UK), glucose oxidase kit (Randox UK), streptozotocin (Sigma-Aldrich, UK), metformin (Merck Sante) suspended in 3 % Tween 80.

Stock solutions of drugs were stored in a refrigerator at 4ºC. All chemicals were of analytical grade.

Animals

Wistar albino rats of both sexes weighing between 200 and 300 g were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. They were allowed free access to water or a particular solution and fed standard diet (Bendel Feeds and Flour Mills Ltd, Ewu, Edo State). Depending on the group, animals were housed 5 in a cage with a 12 hr daylight cycle.

Ethical approval

Approval for the work was obtained from the Faculty of Pharmacy Ethical Committee on the use of animals for experiments, University of Benin, Benin City, Nigeria. The animals were handled according to standard protocols for the use of laboratory animals [5].

Procedure for potassium adaptation

The potassium-adapted rats were given 0.75 % KCl solution orally for 5 weeks in place of tap water [6].

Induction of diabetes

Experimental diabetes mellitus was induced in normal and potassium-adapted male and female adult Wistar rats by intra-peritoneal injection of 60 mg/kg streptozotocin [7] in a 0.1 M citrate buffer solution.

Experimental protocols

Four groups of fasted rats were used, with each group having 5 rats. Group A comprised normal healthy rats and served as control. Potassium-adapted normal rats were also placed in another group (B). Diabetic rats fasted over night (n= 5) were placed in group C. Group D comprised Potassium-adapted diabetic rats. All the groups were administered metformin (350 mg/kg) orally [8] and kept in separate metabolic cages for 24 h after drug administration. Thereafter 24 h urine and blood samples were collected from each rat. The blood samples were collected via cardiac puncture and introduced into lithium heparin bottles. These were spun and the plasma isolated. Plasma samples obtained were then analyzed for glucose, creatinine, triglycerides, total cholesterol, as well as HDL and LDL cholesterol, creatinine clearance and urea. Creatinine and urea in the urine samples were also analyzed.

Statistics analysis

Data are presented as the mean ± standard error of the mean (S.E.M). Comparison of data was made, where appropriate, by one-way ANOVA (GraphPad Prism Software, UK, version 2.05a) with Tukey post hoc. A value of $p < 0.05$ was applied to determine significant differences in all cases.

RESULTS

Effect on lipid profiles

Table 1 shows the effect of potassium-adaptation on the lipid profile of the diabetic rats treated with metformin to be significantly different from the metformin treated group. Treatment of the potassium-adapted diabetic group with metformin significantly increased total cholesterol (TC) and low density lipoprotein (LDL), while triglycerides (TG) were significantly decreased ($p<0.05$). The LDL of the diabetic group (D) was significantly ($p<0.05$) reduced on treatment with metformin.

Effect of potassium-adaptation on creatinine and urea

The effect of potassium adaptation on plasma and urine creatinine and urea (plasma and urine) of diabetic rats treated with metformin are presented in Table 2. The potassium-adapted group treated with metformin (KM) had a significantly ($p < 0.05$) higher urinary creatinine than the non-adapted group, treated with metformin (Met). The plasma creatinine of the diabetic group treated with metformin (DMe) was significantly lower ($p < 0.05$) than those of the diabetic rats (D). Treatment of the potassium diabetic group with metformin (KDM) significantly decreased

Table 1: Effect of potassium adaptation on the plasma lipid profiles of streptozotocin-induced diabetic rats treated with metformin (350 mg/kg).

Treatment	TC	HDL	LDL	TG
C (2ml/kg)	61.81± 3.52	28.59± 3.42	22.86± 2.47	75.20± 10.93
K	44.32± 5.95^{b}	47.68± 1.75^{b}	12.07± 2.80^{b}	26.82± 6.94^{b}
Met	60.86± 1.57	42.94± 2.62^{b}	12.56± 2.38^{b}	26.79± 1.79^{b}
KM	66.00± 1.45 ^{ad}	21.65± 5.84 ^{ad}	37.08± 7.47^{ad}	41.06± 1.48^{ad}
D	60.13± 6.35	20.12± 2.89	33.10± 3.42^{b}	46.55± 7.87
DMe	40.3 ± 4.01	27.41± 3.59	13.13± 2.88^{ab}	58.0 ± 7.95
KD	31.78± 2.54 ^{ab}	26.14± 2.50	-31.56± 4.46^{ab}	186.0± 24.60^{ab}
KDM	42.32± 2.39 ^{ac}	32.33± 4.25	12.23± 2.30 ^{ac}	47.58± 8.36^{ac}

Values are mean ± SEM, n = 5; $^{b}p < 0.05$ significantly different from control; $^{ab}p < 0.05$ significantly different from the diabetic group, $^{ac}p < 0.05$ significantly different from the potassium-adapted diabetic group and $^{ad}p < 0.05$ significantly different from the metformin-treated group.

Table 2: Effect of potassium adaptation on plasma and urinary levels of creatinine and urea of streptozotocin- induced diabetic rats treated with metformin (350 mg/kg).

	Creatinine (mmol/l)		Urea (mmol/l)	
Treatment	***Plasma***	***Urine***	***Plasma***	***Urine***
C (2ml/kg)	0.21±0.03	2.67±0.62	9.27±0.37	785.85±116.90
K	0.09±0.01 [b]	1.77±0.13	9.44±0.42	469.39±62.26 [b]
Met	0.13± 0.01 [b]	11.42± 2.39 [b]	7.23± 0.28 [b]	500.41±103.30
KM	0.12± 0.01	30.25± 0.93 [ad]	25.37± 5.05 [ad]	199.50±58.31 [ad]
D	0.26±0.03	2.64±0.46	11.23±0.49 [b]	473.33±81.50 [b]
DMe	0.14± 0.01 [ab]	3.04±1.08	9.60± 0.50 [ab]	586.54± 113.90
KD	0.45±0.03 [ab]	10.97±1.14 [ab]	12.47±2.35	319.20±51.15
KDM	0.20± 0.01 [ac]	8.54± 0.22	7.06± 0.83	213.49± 37.65

Values are mean ± SEM, n = 5; [a] $p < 0.0001$, [b] $p < 0.05$ significantly different from control; [ab] $p < 0.05$ significantly different from the diabetic group; [ac] $p < 0.05$ significantly different from the potassium-adapted diabetic group; [ad] $p < 0.05$ significantly different from the glibenclamide-treated group.

($p < 0.05$) the plasma creatinine of the potassium diabetic group (KD). The potassium-adapted group treated with metformin had significantly different ($p<0.05$) plasma and urinary creatinine relative to the metformin-treated, non adapted group (Table 2).

Plasma urea in the diabetic group treated with metformin was significantly lower ($p < 0.05$) than those of the diabetics. Treatment of the potassium-adapted diabetic group with metformin did not bring about any significant differences in plasma and urinary urea levels.

Effects on fasting blood glucose and urine volume

The effects of potassium adaptation on the blood glucose and urine volume are presented in Figures 1 and 2, respectively. Treatment of the diabetic rats with metformin (DMe) significantly ($p < 0.05$) lowered their blood glucose levels unlike in the diabetic rats adapted to potassium (KD), where metformin was unable to lower their blood glucose level. It was also observed that the potassium adapted diabetic rats had significantly higher ($p < 0.05$) blood glucose levels, compared with the non-adapted diabetic rats (D).

Treatment of the diabetic and potassium adapted diabetic groups with metformin significantly reduced ($p < 0.05$) urine volume compared with both the untreated diabetic and potassium-adapted diabetic groups, respectively.

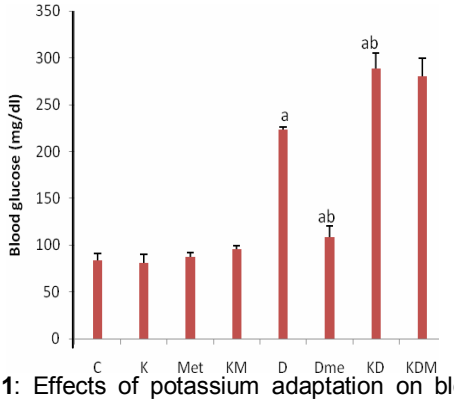

Fig 1: Effects of potassium adaptation on blood glucose of streptozotocin-induced diabetic rats treated with metformin (350 mg/kg). Values are mean ± SEM, n = 5; [a] $p < 0.0001$, [b] $p < 0.05$ significantly different from control; [ab] $p < 0.05$ significantly different from the diabetic group; [ac] $p < 0.05$ significantly different from the potassium-adapted diabetic group; [ad] $p < 0.05$ significantly different from the glibenclamide-treated group

Effect on creatinine clearance

The creatinine clearance of the metformin group was significantly higher than that of the control (Fig 3). The potassium-adapted group treated with metformin had a significantly ($p < 0.05$) higher creatinine clearance than the

non-adapted group treated with metformin. Treatment of the diabetic group with metformin significantly ($p < 0.05$) reduced the creatinine clearance of the diabetics.

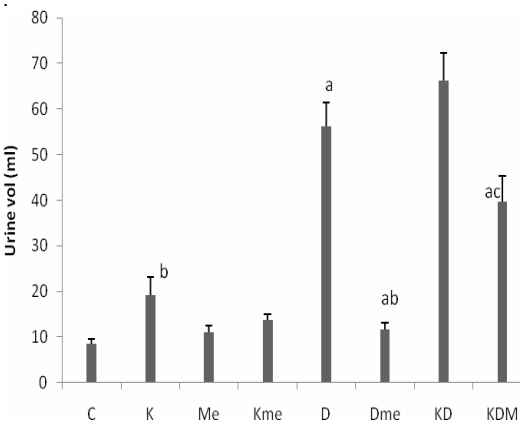

Fig 2: Effects of potassium adaptation on urine volume of streptozotocin-induced diabetic rats treated with metformin (350 mg/kg Values are mean ± SEM, n = 5; $^{a}p < 0.0001$, $^{b}p < 0.05$ significantly different from control; $^{ab}p < 0.05$ significantly different from the diabetic group; $^{ac}p < 0.05$ significantly different from the potassium-adapted diabetic group; $^{ad}p < 0.05$ significantly different from the glibenclamide-treated group.

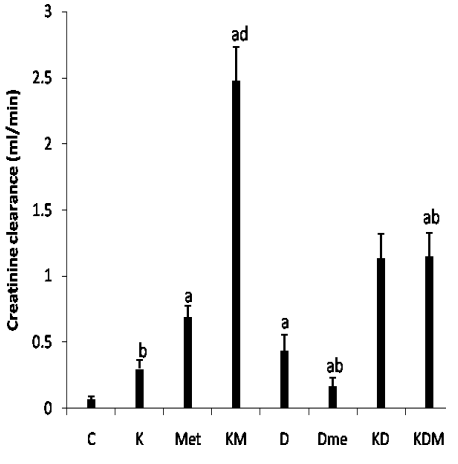

Fig 3: Effect of potassium adaptation on creatinine clearance of streptozotocin -induced diabetic rats treated with metformin. Values are mean ± SEM, n = 5; $^{a}p < 0.0001$, $^{b}p < 0.05$ significantly different from control; $^{ab}p < 0.05$ significantly different from the diabetic group; $^{ac}p < 0.05$ significantly different from the potassium-adapted diabetic group; $^{ad}p < 0.05$ significantly different from the glibenclamide-treated group.

DISCUSSION

Metformin is an oral hypoglycaemic agent that exhibits an antihpyerglycemic effect devoid of insulin release [8]. The blood glucose level of the untreated diabetic rats was significantly reduced on treatment with metformin, however that of potassium adapted diabetics significantly higher than that of the non-adapted diabetic rats were not lowered on treatment with metformin. This seems to suggest that potassium adaptation brings about resistance to the beneficial effects of metformin.

The creatinine clearance of the potassium-adapted diabetic group was not affected by treatment with metformin; however, increase in creatinine clearance was noted for both groups that received metformin (adapted and non-adapted), pointing to the ability of metformin to elevate creatinine clearance. This may point to a possible protective effect of metformin against renal abnormality [9]. The volume of urine for both the diabetic rats and the potassium-adapted diabetic rats were significantly lowered on treatment with metformin. Polyuria is a well known symptom of diabetes [3], effectively controlled on treatment with metformin. From results obtained it is clear that potassium adaptation increases urine output, however treatment with metformin in this group significantly lowered the urine output, to an almost acceptable level (using the control values as the acceptable) especially for the non-adapted diabetics.

The LDL cholesterol of the diabetic group, shown to be raised in this study, was significantly lowered on treatment with metformin. High levels of LDL cholesterol can signal medical problems like cardiovascular disease, hence it is sometimes referred to as bad cholesterol [10]. Treatment with metformin, as this study suggests, would be able to protect against cardiovascular risk by lowering of LDL and raising HDL.

Metformin treatment raised the HDL values in normal, diabetic and potassium adapted diabetic rats in comparison with the control. Increased levels of HDL usually protects against cardiovascular diseases, and low HDL cholesterol levels (< 40 mg/dL) increase the risk for heart disease [12]. As seen in other studies done, our study simply confirms that metformin treatment is thus advantageous to the diabetic because of both the increase and decrease in HDL and LDL respectively.

The high triglyceride value observed in the potassium-adapted diabetic group (186 mg/dl) was significantly reduced on treatment with metformin to 47.58 mg/dl. This again points to a protective effect as high triglycerides are often part of a group of conditions called metabolic syndrome. This syndrome increases the risk for heart disease as well as for diabetes and stroke [12].

CONCLUSION

This study shows that potassium adaptation induces resistance to treatment with metformin in diabetes mellitus, but seems to have an advantage in the treatment of diabetes mellitus in the absence of metformin, as indicated by increase in HDL as well as decrease in both LDL and triglycerides. Metformin may be a good option for diabetes mellitus in the absence of potassium adaptation because of its protective effect against cardiovascular risk.

Conflict of interest

The authors report no conflicts of interest. They are solely responsible for the content and writing of this paper.

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