



Research Articles

Favorable Pregnancy Outcome Following Gliclazide use Throughout Pregnancy in Diabetic Female Sprague-Dawley Rats

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SUMMARY

Objectives: The incidence of diabetes mellitus (DM) is associated with complications with reproduction. The effect of gliclazide on pregnancy hormones and fetal outcomes in diabetic female rats was determined in this study.

Methods: There were four experimental groups (control, diabetic control, gliclazide-treated and diabetic-treated) of 20 rats each. Studies were carried out on days 7, 14, 19 and at term, to ascertain implantation, gestation, fetal outcome and measurement of some plasma hormone levels. Values represent mean $\pm$ SD and were analyzed by two-tailed ANOVA followed by a Post-hoc test. Differences were considered significant at $p < 0.05$.

Results: There were increases in plasma estradiol in the gliclazide-treated non-diabetic [day 14 (55.80 ± 1.0 ; $p = 0.01$), day 19 (55.80 ± 3.1 ; $p = 0.02$)] and diabetic [day 14 (25.42 ± 1.7); $p = 0.03$] groups, and also in plasma progesterone in the treated diabetic group [day 14 (35.12 ± 6.2) $p = 0.02$] only. Plasma corticosterone and plasma CRP decreased in the gliclazide-treated [corticosterone day 14 (356.30 ± 24.1) $p = 0.01$; day 19 (308.30 ± 17.6) $p = 0.01$]; [CRP day 14 (0.13 ± 0.05) $p = 0.01$] and the diabetic-treated [corticosterone day 14 (400.50 ± 21.5) $p = 0.01$, day 19 (424.10 ± 22.4) $p = 0.01$]; [CRP day 14 (0.14 ± 0.13) $p = 0.01$] groups. There were no significant changes in the plasma levels of β -hCG and no abnormalities were observed in litters from all groups.

Conclusion: Gliclazide improved some reproductive complications in the diabetic state and in the non-diabetic state. Consideration should be given to its use in managing reproductive complications associated with DM.

INTRODUCTION

Alterations in carbohydrate homeostasis, such as in diabetes mellitus, have been associated with disturbances of the functional activity of the reproductive system in laboratory animals, not only of the hypothalamo-pituitary axis, but also of the gonad. It can also cause complications during pregnancy, such as spontaneous abortions, recurrent pregnancy loss, preeclampsia and intrauterine growth restriction.[1] Also, women with Type 1 diabetic with initial glycolhemoglobin concentrations in pregnancy above 12% or median first trimester pre-prandial glucose concentrations above 120mg/dl have an increased risk of abortions and malformations.[2] Maternal DM, whether pre-gestational (Type I or II) or gestational in origin or appearance, may create an intrauterine environment of hyperglycemia that promotes fetal insulin secretion and growth beyond the already rapid increase in fetal weight expected during the last trimester of pregnancy.[3] This leads to the higher incidence of macrosomia commonly observed in pregnancies complicated by DM.[4] Several studies have also linked

elevated glucose levels with the development of oxidative stress.[5] Mechanisms involved include glucose anti-oxidation, protein glycation, advanced glycation products and polyol pathway. Oxidative stress, which is caused by an imbalance between pro-oxidants and antioxidants, creates an environment unsuitable for female reproductive function.[6] This can in turn lead to a number of reproductive diseases including endometrioses, polycystic ovary syndrome (PCOS), and unexplained infertility.

Treatment for DM includes, apart from changes in lifestyle, the use of oral antidiabetics. Gliclazide is an oral antidiabetic of the sulphonylurea class. It is used for control of hyperglycemia in gliclazide-responsive DM of stable, mild, non-ketosis prone, maturity-onset or adult type.[7] The non-diabetic effects of gliclazide have been well documented.[7,8]

It possesses chemical properties that could be beneficial in the management of reproductive complications associated with DM and the consequent infertility. But more important are its antioxidant/oxidative effects.[9]

In Africa, there is a lot of stigmatization of women owing to infertility. This leads to personal grief and frustration and serious economic deprivation. The expected increase in the prevalence of DM would further increase the extremely high rates of female infertility already found in Africa. Gliclazide is easily available and inexpensive and could provide possible alternatives to the management of infertility and the associated reproductive complications of DM, thereby improving on social and economic factors of women both in Africa and worldwide. In the light of this, this study seeks to determine the effect of gliclazide on pregnancy hormones and fetal outcomes in normal and diabetic female Sprague Dawley rats and to suggest possible mechanisms by which it could elicit the effects.

MATERIALS AND METHODS

Animals

Adult female rats (120-130g) of the Sprague Dawley strain were obtained from the Animal House of the College of Medicine of the University of Lagos (CMUL). They were kept under a 12hr light:12hr dark cycle and were allowed free access to feed and water throughout the experimental period. Animal identification was carried out in the Department of Zoology, University of Lagos. All guidelines for the use and care of laboratory animals were strictly adhered to in accordance to the CMUL Guidelines on the Use and Care of Laboratory Animals

Animal Grouping

Rats were divided into four groups (n=20): Group I Control (distilled water); Group II - Diabetic Control (distilled water); Group III - Gliclazide (10mg/kg of body weight daily); and (group IV) diabetic treated (gliclazide 10mg/kg of body weight daily).

Drug Preparation and Administration

Gliclazide powder (Sigma-Aldrich Inc, USA) was suspended in distilled water to yield a concentration of 1mg/ml. The dose selected was 10mg/kg of body weight daily.[10] Administration was done once daily and lasted throughout the period of pregnancy.

Induction of Diabetes Mellitus

Diabetes was induced in the morning after fasting the animals overnight by a single intraperitoneal injection of 100 mg/kg of 10% w/v alloxan monohydrate (Sigma-Aldrich, Inc, USA) dissolved in distilled water. After 72 hours, blood glucose level of the dosed rats was checked and those with blood glucose level more than 200 mg/dl were considered, selected and used for the study.[5]

Determination of Plasma Glucose

To assess the effect of alloxan and to chemically establish the diabetic condition, an incision was done on the tip end of the rats' tails, using an insulin needle, seventy-two (72) hours after injection of alloxan. A sample of the rat's blood was collected on a reagent strip to determine the blood glucose level using a portable glucose analyser (Arcu Check Inc., California, USA).

Mating and Induction of Pregnancy

Cycling diabetic and non-diabetic rats in proestrus were isolated and mated on the evening of proestrus. The presence of a sperm plug in the rat vagina (as well as the presence of sperm cells in the vagina smear of the rat) the following morning, confirmed mating. This day was then recorded as day 1 of pregnancy.

Determination of Implantation Sites

Implantation is the stage of pregnancy at which the already fertilized egg adheres to the wall of the uterine horn. To determine the implantation sites, 1mg/ml of Evans Blue was injected via rat tail veins and allowed to circulate for about 15minutes. Animals were then euthanized and dissected; the implantation sites in the fallopian tubes were observed and counted on day 7 of pregnancy.[11]

Studies on Pregnancy and Foetal Outcomes

Fifteen pregnant rats from each group (five for each day) were sacrificed on days 7 (1st trimester), 14 (2nd trimester) and 19 (3rd trimester) of pregnancy, while the remaining rats (five per group) were allowed to deliver. The placental weight, number of foetal resorption and foetal weight were taken and recorded on days 14 and 19. Gestation length, litter size and birth weight were recorded in the rats allowed to deliver. To assess the foetal outcomes, the head length and crown-rump length were measured and the live/birth index calculated. Also, the presence of cleft palate, number of limbs and digits were observed and counted by physical examination.

Determination of Hormone Levels

Enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Co., Ltd, China.) were used for the determination of insulin, oestradiol, progesterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), Creactive peptide (CRP), corticotrophin releasing hormone (CRH) and (beta human chorionic gonadotropin (β -hCG)). ELISA analysis was conducted during the phases of oestrous cycle and in pregnant rats on days 7, 14 and 19 of pregnancy.

Statistical Analysis

Analysis of data was carried out using GraphPad Prism version 6.00 for windows (GraphPad Software, San Diego, California). Results are presented as mean $\pm$ standard deviation. Differences between groups were analysed by two-tailed ANOVA followed by Student's Newman-Keuls post-hoc test. In all statistical analyses, differences were considered significant when p was less than 0.05.

RESULTS

Pregnancy and foetal outcomes

Table 1 shows the effect of gliclazide on pregnancy and foetal outcomes of diabetic female rats.

All of the non-diabetic rats had a positive pregnancy diagnosis. Following initial mating, only 40% of the rats assigned to the diabetic groups (non-treated diabetic = 7 and treated diabetic = 9) had a positive pregnancy diagnosis, with successful pregnancy achieved in the remaining following

subsequent mating. During the entire period of pregnancy, rats in the control and gliclazide-treated non-diabetic groups had normal glycaemic values (80-100mg/dl) and glycemia remained between 200-400mg/dl in the diabetic rats. Percent weight gain during pregnancy in all experimental groups [diabetic control (30.43±2.19; p = 0.02), gliclazide (10mg/kg of body weight) (33.97±4.23; p = 0.03), and diabetic (gliclazide treated 10mg/kg of body weight) (35.77±1.59; p = 0.04)] were significantly reduced when compared to control (43.66±2.95). No significant (p > 0.05) changes were observed in the gestation lengths when the treated groups were compared to their controls. A significant (p = 0.02) reduction was recorded in the number of fetuses in the diabetic group (4.8±0.67) when compared to control (8.5±0.87) with a significant increase (p = 0.04) following treatment with gliclazide (6.6±0.68). The observed changes in the diabetic control when compared to the non-diabetic control was also expressed in the average litter size at birth with a significant (p = 0.01) reduction in the diabetic control [4.9±0.46 (p = 0.01)] when compared to non-diabetic control (8.1±0.25). The highest litter weight was recorded in the diabetic control (6.47±0.18; p = 0.08), while the least was recorded in the gliclazide (10mg/kg of body weight) group (5.78±0.36; p = 0.06). No significant difference was recorded in the head-length and crown-rump length when litters from the treated groups were compared to their controls. No physical abnormalities were observed in the litters and all litters had 20 digits. There was no cleft palate in any of the litters and no other abnormality was observed at birth, two weeks and five weeks after birth. Life-birth index was 100%

for all pregnant rats.

Hormone Levels during Pregnancy

Table 2 shows the effect of gliclazide on hormone levels during pregnancy in diabetic female rats. Significantly elevated (p < 0.05) levels of oestradiol was observed on day 14 (d 14) and day 19 (d 19) of the gliclazide-treated [55.80±2.24 (p = 0.01) (d 14) and 55.80±6.93 (p = 0.02) (d 19)]. The remaining experimental groups did not show significant changes (p > 0.05) when compared to the control [20.00±2.24 (d 14) and 35.00±5.59 (d 19)]. A significant reduction in the plasma level of CRP was recorded on d 14 in all the experimental groups [gliclazide (10mg/kg of body weight daily) - 0.13±0.11 (p = 0.01) and diabetic (gliclazide 10mg/kg of body weight daily) - 0.14±0.29 (p = 0.01)] when compared to control (0.20±0.02) and diabetic (0.22±0.13) groups. All treated groups showed significant reductions (p<0.05) in corticosterone levels on d 14 [gliclazide (10mg/kg of body weight daily) - 356.3±53.89 (p = 0.01) and diabetic (gliclazide 10mg/kg of body weight daily) - 400.5±48.08 (p= 0.02)] and d 19 [gliclazide (10mg/kg of body weight daily)- 308.3±39.36 (p = 0.01) and diabetic (gliclazide 10mg/kg of body weight daily) - 424.1±50.09 (p = 0.02)], when compared to control [491.0±36.0 (d 14) and 475.0±24.18 (d 19)]. Plasma progesterone level was also significantly elevated (p = 0.02) in the treated diabetic group (35.12±6.2). There was no significant change observed in the plasma levels of β-hCG throughout gestation in all experimental groups when compared to control.

Table 1: Pregnancy and foetal outcomes in diabetic rats treated with gliclazide

Groups	Control (drug vehicle)	Diabetic (drug vehicle)	Gliclazide (10mg/kg)	Diabetic/Gli. (10mg/kg)
Gestation length (days)	22.5±0.96	21.8±0.28	20.8±0.33	21.0±0.49
% Weight change in pregnancy	43.6±1.32	30.4±0.98*	33.9±1.89*	32.7±0.71
No. of foetuses (d 19)	8.5±0.39	4.8±0.30*	8.2±0.34	6.6±0.34**
Weight of foetuses (grams) (d 19)	6.26±0.14	6.67±0.09	5.78±0.06	6.30±0.11
Average litter size	8.1±0.11	4.9±0.21*	8.0±0.36	5.97±0.32
Weight of placenta (grams) (d 19)	0.04±0.01	0.06±0.02	0.04±0.01	0.06±0.04
Weight of litters at birth (grams)	6.36±0.11	6.47±0.08	5.78±0.16	6.36±0.11
Head length (cm)	1.92±0.02	1.91±0.03	1.85±0.31	1.92±0.04
Crown-rump length (cm)	5.83±0.12	6.33±0.11	5.52±0.13	6.16±0.09

*p<0.05 when compared to control; **p<0.05 when compared to diabetic control

Bld = Blood; d=day; Glc = glucose; Gli= Gliclazide

Table 2: Hormone levels in control and experimental rats during pregnancy

	Day	Control (drug vehicle)	Diabetic (drug vehicle)	Gliclazide (10mg/kg)	Diabetic/Gli (10mg/kg)
Fasting Blood glc (mg/dl)	1	82.5±1.9	310.5±10.9*	94.8±2.4	260.4±8.2**
	19	87.5±3.1	288.5±18.5*	85.8±3.3	242.9±18.9**
Fasting plasma insulin (µIU/ml)	19	30.0±0.69	11.9±0.35*	28.42±0.82	12.6±0.41
β-hCG (mIU/ml)	7	7.1±0.3	6.2±0.1	5.9±0.4	6.8±0.1
	14	9.0±0.1	8.1±0.7	9.1±0.1	8.5±0.4
	19	8.5±0.2	7.5±1.0	6.7±0.2	7.6±0.5
Corticosterone (pg/ml)	7	427.0±18.0	415.2±14.1	420.1±19.2	416.7±19.3
	14	491.0±16.1	479.1±12.9	356.3±24.1*	400.5±21.5**
	19	475.0±12.6	480.0±15.2	308.3±17.6*	424.1±22.4**
CRP (µg/ml)	7	0.10±0.04	0.12±0.08	0.12±0.06	0.13±0.20
	14	0.20±0.0	10.22±0.06	0.13±0.05*	0.14±0.13**
	19	0.10±0.09	0.15±0.04	0.11±0.05	0.13±0.20
Progesterone (ng/ml)	7	15.00±0.3	13.0±0.2	16.12±0.1	12.11±1.3
	14	35.00±0.6	30.2±2.6	37.50±0.2	35.12±6.2**
	19	60.00±0.4	49.1±1.4	52.50±0.3	48.91±2.4
Estradiol (pg/ml)	7	14.12±1.5	12.11±0.5	19.14±0.9	12.78±1.2
	14	20.00±1.0	21.30±1.3	55.80±1.0*	25.42±1.7**
	19	35.00±2.5	34.56±1.0	55.80±3.1*	39.91±2.7

*p<0.05 when compared to control (non-diabetic) **p<0.05 when compared to diabetic control

CRP- C-reactive protein Gli-gliclazide

DISCUSSION

Maternal weight gain is a component of stored fat, fluid gain, breast tissue, the foetus and the placenta. The least maternal weight gain was seen in the gliclazide treated non-diabetic group. The number of foetuses and litter size were significantly reduced in the diabetic groups. This agrees with studies by Eriksson [12] who reported decreased embryos in diabetic mothers and Ibrahim *et al.*, [13] who reported increased resorption at the uterine horns with a subsequent decrease in litter size of pregnant rats especially with gliclazide treatment.

The highest birth weight was seen in the diabetic untreated group. This is similar to the work of David *et al* [14] who suggested that the increase in birthweight was due to organomegaly secondary to foetal hyperinsulinemia in their study on the effect of maternal DM on the foetus and the neonate. Low birth weight is associated with the risk of adult cardiovascular disease. Evidence exists that foetal adaptation to challenges in the intrauterine environment may adversely affect long-term cardiovascular health. [15] In another report, adaptations to a suboptimal intrauterine environment may also occur, without noticeable reduction in birthweight and still be of long-term importance. [16] The significant reduction seen in birthweight in the gliclazide-treated group could be due to a direct toxicity of gliclazide on the foetus or due to placental dysfunction. [13] This can predispose to possible cardiovascular disease later in life. Ibrahim *et al.*, [13] reported a reduction in foetal weight and foetal anomalies in gliclazide-treated rats, but Yaris *et al.*, [17] reported a normal pregnancy outcome in a diabetic woman following gliclazide administration in the first trimester of pregnancy. The present experiments did not show any malformations (suggested by absence of cleft palates and presence of complete number of digits) in the litters of all experimental groups. This suggests that gliclazide may not have any teratogenic effects.

During pregnancy, levels of oestrogen and progesterone rise steadily, in large part as a result of placental production of these hormones. Levels of human chorionic gonadotrophin (hCG) and cortisol rise across pregnancy, reaching maximum near term and declining after delivery. β-hCG promotes the maintenance of the corpus luteum during pregnancy. Due to its high negative charge, it may repel the immune cells of the mother, protecting the foetus during the first trimester. Though serum levels have been shown to decrease in insulin-requiring diabetic women, [18] the present study did not reveal any significant changes in the level of β-hCG in female diabetic rats following administration of gliclazide during pregnancy. Intrauterine growth retardation increases the risk of neonatal morbidity and is implicated in the progression of adult-onset diseases. Glucocorticoids provide key signals for differentiation of foetal and placental tissues (principally via cortisol in humans and corticosterone in rodents). [19] Yet excess exposure to glucocorticoids limits placental and foetal growth. Findings from the present study showed a significant decrease in the level of corticosterone following gliclazide administration in the normal (non-diabetic) and diabetic pregnant rats. It is possible that gliclazide interrupts the production of 11-β-hydroxysteroid dehydrogenase. This can lead to the decrease in corticosterone levels observed in this study. It is also possible that gliclazide could alter the concentrations of Hexose-6-phosphate dehydrogenase and Isocitrate dehydrogenase. These enzymes are found in the endoplasmic reticulum and regulate NADPH availability, a cofactor required for 11-β-hydroxysteroid dehydrogenase synthesis.

C-reactive peptide (CRP) is a sensitive marker of systemic inflammation. High concentrations of maternal CRP have been associated with adverse pregnancy outcomes and premature contractions may be predicted by elevated levels of CRP. Significantly lowered levels of CRP were observed in the treated groups (both non-diabetic and diabetic). This

implies that gliclazide has both maternal and foetal protective effects during pregnancy.

CONCLUSION

In conclusion, elevated levels of oestrogen and progesterone with a concomitant decrease in CRP levels during pregnancy suggest a maternal and foetal-protective effect of gliclazide. Findings from this study also show that the use of gliclazide in pregnancy is not associated with foetal abnormalities. These suggest that gliclazide improves on some reproductive functions in the diabetic and even in the non-diabetic state. Hence, even though utmost care should be taken before the prescription of gliclazide in pregnancy, more consideration should be given to the non-hypoglycaemic effects of this drug and its possible use in the management of reproductive complications associated with DM.

Conflict of Interest

The authors declare no competing interests.

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