The Effect of Metformin on Serum Levels of FSH, LH, Oestrogen and Progesterone in Diabetic Rats

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Abstract

Diabetes mellitus represents one of the greatest threats to modern global health. It contributes to sub-fertility in females and also impairs normal menstrual cycle and ovulation. The study was designed to investigate the effect of oral administration of metformin on FSH, LH, oestrogen and progesterone in diabetic rats.

Twenty female rats were divided into four experimental groups of five rats each; Group A serves as Control, and received distilled water *ad libitum*, while Groups B, C and D were the experimental groups; Diabetic untreated, metformin treated and Diabetic treated with metformin, respectively. At the end of the experimental period of four weeks, animals in all groups were sacrificed and blood samples were taken for the determination of FSH, LH, oestrogen, progesterone and glucose level.

The result showed significant ($P<0.05$) reduction in FSH and LH levels in all experimental groups when compared to control and significant increases ($P<0.05$) in the progesterone level in Groups B and D when compared to control.

The result suggest that metformin has a direct effect on the ovary and that its effect on insulin-sensitivity may not solely be responsible for its ovulation-inducing effect.

Keywords: metformin, diabetes, oestrogen, progesterone, LH, FSH

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.

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DM 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 in the liver and muscles and suppress hepatic glucose production through the inhibition of gluconeogenesis and glycogenolysis [16]. However, research shows that DM has effect on reproduction; it will affect females prior to their reproductive age. DM contributes to sub-fertility in females, it impairs female fertility as well as impairment of normal menstrual cycle and ovulation. Hence, rising rates of DM suggests high prevalence of infertility [17].

The inhibitory influences of DM on follicular function have been attributed to various portions of the hypothalamo-hypophyseal-ovarian axis, and all three parts of the axis may be affected under different conditions [8].

Insulin resistance has been defined as a state in which a greater than normal amount of insulin is required to elicit a quantitatively normal response [9]. It leads to increased insulin secretion by β-cells and compensatory hyperinsulinemia. Hyperinsulinemia may stimulate the ovaries directly or indirectly through increase in LH secretion and inhibition of IGF binding protein and SHBG synthesis and secretion[1]. This may lead to anovulation, amenorrhea and infertility. Hence, the improvement of insulin sensitivity by insulin sensitizers may be of therapeutic value in the management of clinical manifestation of DM.

Considering the huge resources required for delivering sustainable health care to diabetics and predominant poverty in the Sub-Saharan region, more efforts to elucidate the pathogenic mechanism of DM and accessibility of affordable modalities of disease management is advocated. Advent of new medical technologies and healthy lifestyle could significantly improve the quality of lifestyle for people with diabetes.

One of the drugs used in the treatment of this disorder is metformin (an oral anti-diabetic drug of the biguanide class). It is the first line drug of choice for the treatment of diabetes in particular, overweight and obese people and those with normal kidney function. It is the treatment of choice for patients with non-insulin dependent DM, after dietary manipulation, showing consistent results in terms of glycaemic control [4].

Results of several studies have demonstrated that metformin can induce regular menstrual cycles and increase ovulation in patients with PCOS [10]. The exact mechanism of action of metformin in patients with PCOS is still unknown. However, metformin exerts a systemic action on the regulation of glucose metabolism by insulin [11], and indices of insulin resistance are strong predictors of the efficiency of metformin therapy [11] which suggest that a drug could act to induce ovulation as a result of improved metabolic control [10].

In addition, peripheral effects to metformin have been found to be dependent on or independent of insulin sensitization in several experimental studies, but thus far, the available data has been unable to clarify the exact mechanism by which metformin restores ovarian function [9].

We now report the effect of metformin administration therapy on reproductive /ovarian hormone levels in alloxan induced diabetic rats as a means to further clarify the mechanisms by which metformin restores ovarian function.

Materials And Methods

Experimental protocol:

Twenty female rats (mean weight 150-180g) were maintained under standard laboratory conditions and were allowed free access to food and water *ad libitum*. Animals were divided randomly into four groups. Group A (drug vehicle); group B- non diabetic/metformin (100mg/kg twice daily for 28 days); group C- diabetic (drug vehicle); and group D- diabetic/metformin (100mg/kg daily for 28 days)

Drug, Route and Duration of Treatment:

The drug was prepared with a known mass of metformin powder suspended in distilled water to yield a suspension of 25mg per ml. Dose selected was 100mg/kg (Choi et al; 2006). The suspension was administered by oral cannula for 28 days before sacrifice

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 ≥200mg/dl were separated and used as diabetic in this study.

At the end of the experimental period, rats were fasted for 12 h and sacrificed. Blood was collected by cardiac puncture and transferred into EDTA bottles for hormonal assays'

Biochemical analysis:

The hormonal evaluation consisted of assays of serum FSH, LH, progesterone and oestradiol. All hormone concentration were analysed using the Enzyme-linked immunnosorbent assay (ELISA) kits, (Monobind Inc. Lake Forest, USA). The process involves linking enzymes which generate a colour product when appropriate substrates are added.

Statistical analysis:

All results were expressed as mean ± 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 analysis was performed using SPSS 17.

Results

Mean FSH, LH, oestrogen, progesterone and glucose levels of control and experimental groups are shown in table 1 below. The mean FSH in control group was 1.70±0.10 as against experimental group B (diabetic untreated) 1.02±0.01, group C (metformin treated) 1.25±0.30 and group D (diabetic/metformin) 1.05±0.01. All experimental groups showed significant reductions ($P<0.05$) when compared to control.

The mean LH in control group was 3.12±0.21 as against experimental groups B (2.20±0.05), C

Table1: Effect of Metformin on plasma glucose level, progesterone, oestrogen, LH and FSH

Variable	Control (distilled Water) (n=5)	Diabetic untreated (n=5)	Metformin treated (n=5)	Diabetic/Met (n=5)
Plasma glucose (mg/dl)	139.75±6.20	277.75±38.58	134.85±5.11	162.75±5.97*
FSH	1.70±0.10	1.02±0.01**	1.25.±0.30**	1.05±0.01**
LH	3.12±0.21	2.20±0.05**	0.95±0.01**	2.25±0.06**
Oestrogen	140.00±11.40	119.00±1.83	120.00±13.1	125.00±6.19
Progesterone	0.58±0.09	2.43±0.15**	0.70±0.11	2.75±0.19**

Values are expressed as mean ± SEM, ** $P<0.05$ when compared to Control, * significantly reduced when compared to Diabetic untreated (Group B)

(0.95±0.01) and D (2.25±0.06). All experimental groups showed significant reductions (P<0.05) when compared to control, with group C (metformin treated) showing the greatest decrease.

The mean oestrogen level in control group was 140.00±11.40 as against experimental groups B (100.00±1.83), C (120.00±13.1) and D (94.00±6.19). All experimental groups showed a reduction in oestrogen level but these changes were insignificant (P>0.05).

Progesterone levels in groups B (diabetic untreated) 2.43±0.15 and D (diabetic/metformin) 2.75±0.19 show significant increase (P<0.05) when compared to control, while group C shows an insignificant reduction (P>0.05).

Discussion:

Drug interactions are usually seen in clinical practice and the mechanism of interactions are usually evaluated in animal models. We studied the influence of metformin on FSH, LH, Oestrogen and Progesterone in normal and diabetic rats. The normal rat model served to quickly identify the interactions and diabetic rat model served to validate the same response in the actually used condition of the drug.

Metformin is an anti-diabetic drug that increases glucose utilization in insulin-sensitive tissues. In women with PCOS, metformin treatment reduces hyperinsulinemia, corrects menstrual irregularity in majority, results in higher ovulation rates, independently of changes in body weight [2]. As PCOS and diabetes share some altered parameters such as abnormal glucose-insulin ration altered lipid metabolism and insulin-resistance syndrome [4], the use of metformin in diabetes may also have some effects in modulating the secondary infertility that can be caused by this disease.

The systemic effect of metformin has been demonstrated extensively, in fact, several experimental and clinical studies most of them translated from a population of patients with type 2 diabetes mellitus, have shown that this drug acts by systemic effects on hepatic gluconeogenesis and the production of SHBG, intestinal free fatty acid oxidation and glucose use and the utilization of glucose by peripheral tissues [13].

Our study showed significant decrease (P<0.05) in FSH and LH levels in the metformin treated group when compared to the control but changes in the levels of the same hormones where not noticeable when the diabetic treated rats were compared to the diabetic untreated rats. This is in agreement to findings by [15] who observed changes in the hormonal levels following induction of ovulation with metformin and suggested that the efficacy of metformin in inducing ovulation is probably due to a direct action of the drug on the ovary, and that the ovulatory response to the drug seems to be related more to local drug sensitivity or resistance than to improvements in the systemic hormonal and /or metabolic pattern.

Although there was a significant reduction (P<0.05) in FSH and LH levels in the diabetic rats, similar to other findings [8,7,3]; this can be explained by the probable negative feedback mechanism that could have been caused by the significantly raised progesterone levels. Oestrogen in small amounts has a strong effect in inhibiting the production of both LH and FSH. Also, when progesterone is available, the inhibitory effect of oestrogen is multiplied, even though progesterone by itself has little effect. The feedback effects seem to operate mainly directly on the anterior pituitary gland but to a lesser extent on the hypothalamus to decrease secretion of GnRH, especially by altering the frequency of the GnRH pulses. This also further explains a possible mechanism by which anovulation complicates diabetes mellitus. The decreased levels of LH can interfere with the LH-surge required for ovulation to occur. Without the initial preovulatory surge of LH, ovulation will not occur. This can lead to anovulation, amenorrhoea and infertility.

In conclusion, our study has shown and further affirms that the ovulation-induction effect of metformin is probably due to its direct effect on the ovary and not solely to the systemic effect of the drug on insulin sensitivity and / or hyperandrogenism.

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