EFFECTS OF VILDAGLIPTIN ON THE PITUITARY– GONADAL AXIS OF MALE WISTAR RATS

By

AZEEZ Taoreed Adegoke

MB.,Ch.B. Ife

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CERTIFICATION

This is to certify that this research work was carried out by AZEEZ Taoreed Adegoke with the registration number BMSP12/13/H/0918, under our close supervision in the Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

……. …….

Dr. O. S. Akinsomisoye (Supervisor)

Dr. A. R. Owolabi (Co-supervisor)

…….

Dr. O. Ogunlade

(Ag. Head, Department of Physiological Sciences, OAU, Ile-Ife).

DEDICATION

This work is dedicated to God, the Pedestal of my existence and subsistence. He has created me specially and has enabled me to accomplish this enormous task. He has turned a creature into a creator; creativity without an end. To Him alone be all the glory.

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Abstract

This study investigated the effects of daily administration of Vildagliptin (an oral anti-diabetic drug) for eight weeks on some sperm parameters, pituitary as well as gonadal hormones, litter size and testicular with epididymal histology of male Wistar rats.

A total of 60 male and 40 female Wistar rats weighing 120 - 150 g were used for the study. The 60 male rats were divided into 3 equal categories (A, B and C). Each category was further divided into 4 groups (making a total of 12 groups), with each group containing 5 rats. In category A, Group 1, the control group, received 1.4 ml/kg of distilled water daily for 8 weeks; while Groups 2, 3, and 4 received 0.35 mg/kg, 0.70 mg/kg and 1.4 mg/kg of Vildagliptin (orally) daily for 8 weeks respectively. Thereafter, the rats were sacrificed to determine the following parameters: sperm characteristics (count, motility, viability and morphology), serum testosterone, follicle-stimulating hormone and luteinizing hormone concentrations. Histology of the testis and epididymis was done. In category B, Group 5, the control group, received 1.4 ml/kg of distilled water daily for 8 weeks via oral route; while Groups 6, 7, and 8 received 0.35 mg/kg, 0.70 mg/kg and 1.4 mg/kg of Vildagliptin (orally) daily for 8 weeks respectively. Thereafter, each male rat was allowed to cohabit (so as to mate) with 2 apparently healthy non-pregnant female rats in separate cages. The litter sizes were determined and summed up for each group. In category C, Group 9, the control group, received 1.4 ml/kg of distilled water daily for 8 weeks; while Groups 10, 11, and 12 received 0.35mg/kg, 0.70 mg/kg and 1.4 mg/kg of Vildagliptin (orally) daily for 8 weeks respectively. All the rats were allowed another 8 weeks of drug-free recovery period. Subsequently, they were sacrificed and the same parameters (as listed for category A) were determined.

The results of the study showed a dose-independent but partly reversible significant decrease in the sperm counts (X 10^6 /ml) of all the Vildagliptin-treated groups when compared with the control ($F=3.89$, $p=0.045$). There was a dose-independent but reversible significant reduction in the sperm motility (%) of the treated groups when compared with the control ($F=9.84$, $p=0.0015$). There was a dose-independent but reversible significant increase in the percentage of sperms with abnormal morphology in the treated groups when compared with the control ($F=4.39$, $p=0.026$). However, there was no significant change in sperm viability ($F=1.00$, $p=0.43$). There was a dose-independent significant decrease in serum testosterone ($F=4.51$, $p=0.040$) and significant increase in serum FSH ($F=4.39$, $p=0.037$) as well as delayed significant increase in LH ($F=4.39$, $p=0.037$) of the treated rats when compared with the control. There was a highly significant dose-dependent decrease in the litter size of treated rats when compared with the control ($F=18.66$, $p<0.0001$). There was no visible deleterious effect on the histoarchitecture of the testes of the treated rats, however there was a dose-dependent distortion in the histoarchitecture of the epididymis of the rats in the treated groups when compared with the control and these were largely reversible after 8 weeks of recovery.

In conclusion, Vildagliptin adversely affected the reproductive structure and function of male Wistar rats. It caused significant deleterious effects on the sperm counts, motility, morphology, epididymal histology as well as the serum testosterone, FSH and LH with resultant decrease in litter size. Further studies will be required to characterize these effects at the molecular level and to devise the means of mitigating the effects.

CHAPTER ONE

1.0 INTRODUCTION

1.1.0 Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases in which there are high blood glucose levels over a prolonged period of time due to deficiency in insulin secretion and/or action (WHO, 2014). Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (Alberti and Zimmet, 1998). If left untreated, diabetes mellitus can cause many complications. Acute complications include diabetic ketoacidosis and hyperglycaemic hyperosmolal state (Kitabchi *et al.*, 2009). Serious long term complications include cardiovascular diseases, chronic kidney disease, foot ulcers and damage to the eyes.

There are 4 types of diabetes mellitus: type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes mellitus and other specific types (ADA, 1979). Adopting a healthy lifestyle is a critical part of delaying or preventing the onset of diabetes. However, for those already diagnosed with the disease, keeping it under control is very important. In many cases, medications are integral parts of the management.

In 2014, the global prevalence of diabetes was estimated to be 9% among adults aged 18 and above (WHO, 2014). In 2012, an estimated 1.5 million deaths were directly caused by diabetes (WHO, 2012). More than 80% of diabetes incidence occur in low-income and medium-income countries (WHO, 2012). The World Health Organization projects that diabetes mellitus

will be the 7th leading cause of death in 2030 (Mathers and Loncars, 2006). 50% of people with diabetes mellitus die of cardiovascular diseases (Morrish *et al.*, 2001).

Insulin is a hormone that regulates blood glucose, among other things. Hyperglycaemia is a common effect of uncontrolled diabetes and over time, it leads to serious damage to many of the body systems, especially the nerves and blood vessels. Impaired glucose tolerance and impaired fasting glucose are intermediate conditions in the transition between normality and diabetes mellitus (NICE, 2012). People with these conditions are at high risk of progressing to type 2 diabetes although this is not inevitable. Early diagnosis can be achieved through relatively inexpensive blood testing. Treatment of diabetes involves lowering blood glucose and the level of other known risk factors that damage blood vessels and nerves.

1.2.0 Antidiabetic drugs

Antidiabetic agents consist of any of the several drugs that are used to control the level of glucose in the blood. They are developed to stabilize blood glucose levels in people with diabetes mellitus. Except few ones like insulin, exenatide and liraglutide, they are mostly administered orally hence, the term 'oral antihyperglycaemic agents' (Koski, 2006).

There are different classes of antidiabetic drugs and determining which one to use depends on the nature of the diabetes, age, severity of the illness, as well as other factors (Harrigan *et al.*, 2001). The main classes are heterogeneous in their modes of action, safety profiles and tolerability (Krentz and Bailey, 2005). When choosing an agent, it is prudent to consider both patient-specific and drug-specific characteristics (Luna and Feinglos, 2001). The various classes of antidiabetic drugs include insulin, biguanides, thiazolidinediones, sulfonylureas, meglitinides, alpha-glucosidase inhibitors, glucagon-like peptide agonists,

dipeptidyl peptidase-4 inhibitors, amylin analogues and sodium-dependent glucose co-transporter-2 (SGLT-2) inhibitors (Moses *et al.*, 2014).

Each class has its own mechanism of action, although some mechanisms of actions are interrelated. If adequate glucose control is not attained using a single oral agent, a combination of agents with different mechanisms of action may have additive therapeutic effects and result in better glycaemic control (Luna and Feingos, 2001).

Insulin injection serves as a replica of endogenously-produced insulin. So, they have the same mechanism of action which involves uptake of glucose by the insulin-sensitive cells (Alegbejo *et al.*, 2014). Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery including the skeletal muscle. Thiazolidinediones bind to peroxisome proliferator-activated receptor gamma (PPAR-γ), a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism (Bermudez *et al.*, 2010).

Sulphonylureas trigger insulin release by inhibiting a specific type of potassium channel on the pancreatic beta cells. Meglitinides act on the same potassium channel as sulfonylureas, but at a different binding site. Alpha-glucosidase inhibitors slow the digestion of carbohydrate in the small intestine thereby allowing glucose to enter into the blood stream more slowly (Osadebe *et al.*, 2014). Glucagon-like peptide-1 agonists bind to a membrane glucagon-like peptide-1 receptor thereby increasing the release of insulin from beta cells. Amylin analogues slow gastric emptying and suppress glucagon secretion. Sodium-dependent glucose co-transporter-2 (SGLT-2) inhibitors block the re-uptake of