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Diabetes mellitus- induction: Effect of different streptozotocin doses on male reproductive parameters

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ABSTRACT

Diabetes mellitus (DM) is reported to be involved in male reproductive impairment, and its impact is evident in the increased prevalence of infertility. Various studies have reported that a single parenteral injection of < 40 mg/kg Streptozotocin (STZ) is ineffective in ablating pancreatic β-cells and creating a rat model to investigate the effect of DM on the male reproductive system. This study therefore aims to validate these claims.

Adult male Wistar rats received either a single intraperitoneal injection of STZ (30 mg/kg or 60 mg/kg) or saline (0.9%, Control). Diabetes was confirmed after 72 h if plasma glucose levels were ≥14 mmol/L. Body weight, glucose level, fluid and food intake were measured weekly. Animals were sacrificed after 8 weeks of treatment by an overdose of sodium pentobarbital (160 mg/kg body weight). The testis and epididymis were harvested and weighed prior to preparation for histological evaluation. Epididymal sperm morphology was analysed using computer aided sperm analysis (CASA). STZ60 animals presented with significantly lower body weights compared to both control and STZ30 groups. Animals in both STZ30 and STZ60 groups showed decreased normal sperm morphology compared to control. Histological evaluation of the testes showed a decrease in the number of spermatozoa in the seminiferous tubules of animals in the STZ30 and STZ60 groups compared to control. A complete absence of spermiogenesis was observed in the seminiferous tubules of STZ60 animals. These findings prove that an STZ concentration of 30 mg/kg, which is much lower than the reported 40 mg/kg, has adverse effects on the male reproductive system via its diabetogenic effect and can therefore be used to study the impact of DM on male fertility.

1. Introduction

Streptozotocin (STZ) is a chemical compound exhibiting antibiotic and antineoplastic properties, and it is produced by the bacterium Streptomyces achromogens (Vavra et al., 1959). Since the diabetogenic introductory report on STZ in 1963 (Rakieten et al., 1963), several work has been done to showcase its mechanism of action and also why it damages the pancreatic β-cells. STZ has a dynamic chemical structure that contains deoxy- glucose molecules which allows it to identify the GLUT2 receptors that is ample on the plasma membrane of pancreatic β- cells (Lenzen, 2008). These molecules are also associated to a highly reactive methylnitrosourea group, which is responsible for the cytotoxic effect of STZ.

Due to STZs high affinity for binding to the glucose receptors present on the pancreatic β-cells, its ingestion relish the cytotoxic effect upon these cells and lead to dysfunction or cell death. This subsequently leads to alteration of insulin levels and blood glucose concentrations (Wei et al., 2003), thereby indicating that STZ may be a suitable diabetes inducing agent. To further validate its potency, Eleazu et al. (2013) reported that there is a rapid elimination of its components by the liver and that any functional impairment observed may be ascribed to the hyperglycaemic effect (Eleazu et al., 2013). In lieu of this, STZ can therefore be used to induce experimental Type I or Type II diabetes mellitus, either independently, or in combination with high fat diet and/or in combo with nicotinamide administration (Skovso, 2014; Szkudelski, 2012).

Diabetes mellitus (DM) is a chronic non-communicable disease that is characterized by hyperglycaemia, which can result from a lack of insulin secretion by the pancreatic β-cells or from target tissues becoming insensitive to insulin. It can be classified into two broad categories: (i) Type I DM, also called insulin-dependent diabetes mellitus (IDDM), is caused by lack of insulin secretion; (ii) Type II DM, also called non-insulin-dependent diabetes mellitus (NIDDM), is caused by the decrease in sensitivity of the target tissues to the metabolic effects of insulin. DM have been shown to affect male reproductive function at multiple levels as a result of its effects on the endocrine control of the

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hypothalamic-pituitary gonadal (HPG) axis, spermatogenesis itself or by impairing penile erection and ejaculation (Sexton and Jarow, 1997). In experimental animals, STZ induced diabetes have been reported to cause decreased serum testosterone, decreased serum FSH and decreased serum LH levels as well as a decrease in the total number of Leydig cells (Ballester et al., 2004; Murray et al., 1985). Another study done on the inhibition of glucose-stimulated insulin secretion in rats' islets of Langerhans by STZ revealed that the main reason for the STZ-induced β-cell death is the alkylation of DNA (Szkudelski, 2001). Shrilatha (2007) further reported increased sperm DNA damage and decrease in sperm count of STZ induced diabetic animals (Shrilatha, 2007). Additionally, Ganda et al. (1976) reported that the use of a single intravenous or intraperitoneal dose in adult rats to induce Type I DM is between 40 and 60 mg/kg body weight (Ganda et al., 1976). Katsumata and Katsumata (1992) supported the above findings and further added that a single dose less than 40 mg/kg body weight may be ineffective. Furthermore, Vikram et al. (2008) reported a biochemical alteration in rat accessory sex organs after administration of STZ at 45 mg/kg body weight Also, Navarro-Casado et al. (2010) reported a decrease in sperm motility, testicular and epididymal weight, following administration of STZ at both 45 mg/kg and 60 mg/kg to investigate its effects on male reproductive function of rats. However, we could not find any study that investigated the effects of a lower dose of STZ on male reproductive function in experimental rats. Therefore, this study was designed to investigate the effects/impact of a lower dose of STZ on male reproductive function and to compare these outcomes with the effects of a known efficacious dose in Wistar rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (240–290 g) were housed in standard ventilated cages, and were exposed to a 12 h light: 12 h dark cycle at 23 °C ± 2 °C. Animals had free access to food (standard Epol™ rat chow) and water. Ethics approval for this research was obtained from the Stellenbosch University Animal Ethics Committee.

2.2. Diabetes induction

STZ (S0130-IG) was obtained from Sigma and prepared by dissolving it in freshly prepared sodium citrate buffer at a pH 4.5 to make a 30 mg/ml stock of STZ. The STZ solution was immediately injected intraperitoneally and administered at doses of 30 mg/kg body weight or 60 mg/kg body weight. The injection volume was calculated according to the dose required and the weight of the animal.

2.3. Experimental design

Animals were randomly assigned to treatment groups to avoid bias and were acclimatized for two weeks. Fifteen rats were divided into three (3) groups (n = 5), namely, a control group and two treatment groups receiving STZ at either 30 mg/kg body weight (STZ30) or 60 mg/kg body weight (STZ60). Animals in the control group were treated with vehicle (0.9% saline). The successful induction of diabetes was confirmed after 72 h, with animals showing a justifying blood glucose level of > 14 mmol/L, using a Glucoplus™ glucometer (Ayeleso et al., 2014; Dludla et al., 2014). The diabetic state was confirmed throughout the 8 weeks treatment period and glucose levels were measured weekly in all groups. The fluid and food intake of the animals were also measured weekly, while the body weights were measured at start and end of the experiment. The relative testicular weight is the sum of both testes divided by the final body weight of the specific animal multiplied by 100. Values are expressed as percentage of body weight.

2.4. Experimental procedure

Rats were humanely treated at all times and sacrificed after 8 weeks of treatment, by an overdose of sodium pentobarbital (160 mg/kg body weight). The testis and epididymis were harvested, rinsed and weighed. The testis was fixed in 10% formalin solution for histological evaluation. The Cauda area of the epididymis was isolated and spermatozoa retrieved, which was subsequently used for motility and morphological analysis.

2.5. Histology

The testicular tissues were fixed in a 10% formalin solution. The tissues were kept in the solution for 48 h minimum to allow for complete fixation. The fixated tissues were dehydrated with series of alcohols, cleared with xylene and infiltrated with paraffin wax. Tissues were processed using automated processor (Duplex Processor, Shandon Elliot).

Processed tissue pieces were embedded in paraffin wax at 60 °C using the Leica EG1160 embedder. Processed tissue blocks were kept at 20–25° C until sectioning. Sections were placed in a water bath (approximately 40° C), allowing the tissue to stretch. Tissues were stained with Haematoxylin and eosin (H & E) using an Auto stainer (Leica Auto stainer XL). Stained tissue slides were mounted with DPX mounting medium and left to dry for 48 h; allowing for proper visualization.

2.6. Histological evaluations

Descriptive and quantitative histological evaluations of the testes were carried out. The quantitative aspect involves the histomorphometric analysis of the seminiferous tubules using the ZENlite histological analyser. This method involves measuring all seminiferous tubules seen in a specific field at a given time point. Seminiferous tubules were measured randomly at x10 magnification and field area 234.3713 mm^2, knowing that all seminiferous tubules cannot be in the same stage of spermatogenesis at the point of analysis and that seminiferous tubules have different sizes at different stages. The following parameters were measured; seminiferous tubule (Tubular) diameter (Fig. 1 yellow line); tubular area (Fig. 1 blue spine contour), epithelial height (Fig. 1 red line) and luminal diameter and area (Fig. 1 black line and green spine contour respectively).

The descriptive analysis was performed by randomly counting 100 seminiferous tubules across different fields (covering the whole section) of the testis sections for each rat with x20 and x40 magnification. Seminiferous tubules were analysed at field area 58.649 mm^2 evaluating maximum of 2 tubules per field to avoid bias and inaccurate analysis. In each animal the tubules were examined and categorized as normal, atrophic, sloughing or undergoing cellular degeneration. Tubules classified as normal are characterized by presenting with the complete spermatogenic phases, normal cellular organization, typical cellular association and regular interstitial spaces. Atrophy represents tubules with very few or absence of germ cells and cellular disorganization, while sloughing is characterized by the immature accumulation of cells in the lumen. Cellular degeneration is characterized by absence of some or all of the spermatogenic phases as well as degeneration of cells (presence of vacuole).

2.7. Evaluation of sperm parameters

2.7.1. Sperm retrieval

The Cauda area of the epididymis was extracted inside a separate HAMs solution (2 ml) forming a sperm solution.

2.7.2. Motility

Sperm motility was analysed through computer aided sperm analysis (CASA) using the Sperm Class Analyzer (SCA, Microptics,