Histological study of testis tissue of male golden hamster 
(*Mesocricetus auratus*) in different time points of Diabetes

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Abstract
Previous studies have reported that hyperglycemia can induce cell death in many tissues like brain, liver, kidneys, and testis. Recent studies have indicated that diabetes can trigger male infertility. In this study we explain the histological analysis of testicular tissue after induced diabetes in hamsters.

Testicular tissues were examined in the control group and in diabetic hamsters groups at different time periods after diabetes induction. Hyperglycemia was induced in experimental male hamsters by intraperitoneal injection of streptozotocin drug (STZ). At different time points (4, 8 and 20 weeks) after diabetes induction, hamsters were euthanized and the testicular tissues were removed for the histological analysis.

Testis tissues were fixed in formalin (10%) and then processed for histological analysis, and examined under light microscope.

The histological results showed reduction in cell density in the testis, which indicated that diabetes and hyperglycemic conditions defect normal cell density in the testis tissues. The testicular histology of the diabetic animals showed acute reduction in cell density occurred after 20 weeks.

In conclusion, the induced diabetes condition provides evidence that hyperglycemia plays an important role in the pathogenesis of diabetes, and it also indicated that chronic hyperglycemia eventually leads to cell death and then male infertility.

Keywords: diabetes, testicular tissue, streptozotocin, histology


Introduction

Diabetes Mellitus has been associated with the reproductive dysfunction in both men and women as all (1). Although the pathophysiology of the reproductive derangements in the young diabetic women has been investigated (2), a few studies have been conducted in men. The defective spermatogenesis may be the consequence of direct testicular effect from the diabetes condition (3). Cell apoptosis in the testicular cells is an important eventof the diabetes and hyperglycemia conditions. The mechanisms that cause the inflammation and apoptosis are beginning to emerged. Although many studies have reported the roles of the diabetes in infertility of male (4) but the morphological changes and the time courses of the cell death after diabetes type 1 induction, have not been reported so far. The reduction of the tissue cell density after the diabetes induction was a major factor in infertility (5). Several mechanisms were proposed for the testicular cell apoptosis and cell death in the diabetes. For example, hyperglycemia can induce reactive oxygen species (ROS) production that leads to the cell apoptosis in various body tissues such as testis (6). These defects are related to the early apoptosis in the diabetic
patients (7). In this study, we evaluated the effects of the chronic hyperglycemia on the morphological cell density of the hamsters testes tissues in various time periods after the diabetes induction.

Materials and Methods

Animals

Adult hamsters (8-6 weeks old with weight 250-200 g). They were housed and maintained at a constant temperature at 20-22°C, with a relative humidity at 55% and standard 12:12 h light-darkness cycles, and they had a free access to standard ad libitum and tap water, and allowed for 1 week acclimatization to the laboratory conditions before the experiment. Animals were injected intraperitoneally with a single dose of streptozotocin drug (STZ; Sigma-Aldrich, Germany) at 55 mg/kg of body weight, drug dissolved in 10 mM of sodium citrate buffer (pH 4.5) after 12 h of food withdrawal. Hamsters injected with citrate alone without STZ were served as the normal control. On the day 2 after STZ induction, a blood sample was obtained from the hamsters vein, for the random glucose levels. At the present study, hyperglycemia was defined at a blood glucose level of 20 mM or higher than that. The citrate buffer treated hamsters were used as a normal glycemic control (blood glucose <12 mM). Groups of 3 diabetic hamsters were sacrificed after diethylether anesthesia at weeks 4, 6, 8, and 20 weeks (late phase of diabetes progression) and the testis tissues were separated for the histological analysis. Three control hamsters in each time point were also sacrificed and then studied. At a specified time points (4, 6, 8, and 20 weeks) post diabetes induction, and also in control groups, hamsters were euthanized, and after the laparatomy, testis tissues were dissected. The testicular samples were immediately fixed at the room temperature in formalin (10%) for the histological examinations. The tissues were processed according to the routine program of a histological tissue processor, and the paraffin blocks were prepared. The specimens were cut at 4 μm-thick sections by the rotary microtome, and mounted on the gelatin coated glass slides. Testis sections (4μm) were deparaffinized in the xylene, rehydrated in decreasing concentrations of ethanol alcohol, and were stained with hematoxyline-eosin stain (HE) and then examined under the light microscope for the evaluation of testicular cell density.

Results

Diabetes was induced in hamsters to assess if high blood glucose can affect the morphological testis cell density. Hamsters with blood glucose >250-300 mg/dL were defined as diabetic animals. Hamsters treated with STZ were showed a typical symptoms of diabetes, such as assignificant hyperglycemia (blood glucose ≥250mg/dL), low body weight compared to the normal controls.

Histological sections of the testis were taken from the hamsters at a specific time points (4, 6, 8 and 20 weeks) after STZ diabetes induction in comparison to the control group revealed progressive reduction of cell density in many specific times after the diabetes induction. These changes were not present in the control hamsters. Testes tissue sections staining with H & E stain from adult diabetic hamsters demonstrated a general structural changes and a gradual reduction in the testicular cell density at different time points (4, 6, 8, and 20 weeks) after the diabetes induction.
Figure 1: Body weight of the studied groups controls and diabetic animals at different time points after diabetes induction.

Figure 2: show seminiferous tubule of controls the testicular cells were appear at normal density and sperm were fills the tubule lumen (H&E stain 10X)

Figure3: show seminiferous tubule of 4 week diabetes, the testicular cells were appear at a density lower than controls (H&E stain 10X)
Figure 4: show seminiferous tubule of 6 week diabetes, the testicular cells were appear at a density lower than controls (H&E stain 10X)

Figure 5: show seminiferous tubule of 8 week diabetes, the testicular cells were appear at a density lower than controls (H&E stain 10X)

Figure 6: show seminiferous tubule of 20 week diabetes, the testicular cells were appear with reduced density and separated from one another (H&E stain 40X)
Discussion

The current study was designed to evaluate the effects of induced hyperglycemia condition (diabetes type 1) following STZ administration on the morphological structures of testis tissues of the male hamsters. Type 1 diabetes is an organ specific autoimmune disease that results from T-cell mediated destruction of the insulin producing pancreatic beta cells in the genetically predisposed individuals (7). For the induction of hyperglycemia and diabetes type 1, we used STZ drug. The major effects of STZ drug is on the pancreatic Langerhans β cells. Results of blood glucose determination showed that blood glucose levels in diabetic groups increased compared to non-diabetic groups. Elevated blood glucose is a result of insulin secreting beta cells destruction and reduction in insulin levels. Histological studies in adult diabetic hamsters showed a testicular cell density reduction. The cell densities were declined in various times after the diabetes type 1 induction, especially after the 20 weeks.

One of the suggested mechanisms of testicular cell density reduction is the over expression of the apoptosis and the proinflammatory mediators in the testicular tissues, which can stimulate the cell death (8). Therefore, the size and the volume of the testis tubules and hence testis volume are decreased in all the diabetic groups. Diabetic changes such as cellular microenvironment, and leads to numerous unwanted effects (9). Many studies have shown that the inflammations induced by hyperglycemia are the main mechanisms of the pathogenesis of cell, the inflammations has a prominent role in apoptosis at various organs in hyperglycemic conditions (10). Innate immune system plays a role in the over expression of the proinflammatory cytokines in diabetic condition (11). The inflammatory cell related apoptosis contributes to the organ damage and the macro and micro vascular complications (12). Also, few studies were reported that innate immunity has a tight correlation with the apoptosis and cell death (13).

It is demonstrated that in the male infertile patients, the oxidative damage has a critical role in the cell apoptosis (14). Numerous cellular and molecular mechanisms were proposed for the cell apoptosis in the diabetic subjects. Previous studies indicated that diabetes induces an advanced glycation end products (AGEs). AGEs contributed to the reactive oxygen species (ROS) production that lead to the oxidative stress and then cell death (8). Also, AGEs in diabetic conditions induced an inflammation response via over expression of the proinflammatory cytokines and chemokines for instance TNF-α and IL-1β in the various cells especially monocytes and macrophages (15). The proinflammatory cytokines increased the endothelial permeability and induced leukocyte adhesion to the vascular endothelium (15). The proinflammatory secretion by leukocytes leads to the destruction of various tissues in the diabetic subjects. The upregulation of the proinflammatory cytokines following the hyperglycemia and activates the nuclear factor kappa B (NF-κB) which translocated from the cytoplasm to the nucleus, and regulated the overexpression of the inflammatory response and leading to the cell impairment and then apoptosis. Also, the proinflammatory cytokines such as IL-1β can trigger the overexpression of the Fas and induce Fas-mediated apoptosis (11). Another mechanisms were also proposed for the cell apoptosis in the diabetic conditions.

The main target organ that affected after the diabetes is the testis. Some previous studies were reported that the diabetes can affected kidneys (16), so, hyperglycemic conditions could induced apoptosis in the renal cells and cause the diabetic nephropathy (17). In addition to the hyperglycemia, other factors like the fatty acids can contributed in the pathological aspects of diabetes. For example, the ectopic lipid accumulation could initiate the cellular apoptosis and the testicular dysfunction (17). Several studies were reported that high concentration of glucose can induce the ROS and the proinflammatory mediators in the diabetic conditions (15).

Our recent results showed that diabetes type 1 could leads to the reduction of cell density and increased apoptosis in the testis tissue in various time points after the diabetes type 1 induction. The highest reduction of the cell density was observed after 20 weeks of diabetes.

Conclusion

Hyperglycemia may undesirably cause cell death and lead to the cell apoptosis in the testicular tissues through creating the AGEs, inducing proinflammatory responses, which leads to the impairment of spermatogenesis and then the male infertility.

References


