A HISTOLOGICAL STUDY OF THE STRUCTURAL CHANGES IN THE PANCREAS OF DIABETIC RATS*

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ABSTRACT

The present study was aimed to demonstrate the histological changes in the pancreas of streptozotocin (STZ) induced diabetes in a rat model. Twenty rats were randomly separated into two groups of ten rats each. Group I served as normal control and group II served as diabetic. The diabetic condition was induced in group II by streptozotocin. Light microscopic evaluation of islets showed highly swollen β-cells with loss of cytoplasmic granularity, vacuolations, necrosis, elongated and fusiform β-cells and sparse cellularity in diabetic rats. The present study showed that induction of diabetes using STZ results in the alteration of the morphology of endocrine part of pancreas in rats.

Keywords: β-cells, Diabetes Mellitus, Necrosis, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a major health problem worldwide. The economic impacts of diabetes with its complications and associated diseases are large (Sarah *et al*., 2004). According to recent estimates, the prevalence of diabetes mellitus is 4 percent worldwide and that indicates 143 million persons are affected which will increase to 300 million by the year 2025 (Analava *et al*., 2007).

Animal models in diabetes research are very common where rats are the first choice of use, comprising over 85 percent of these models (US Department of Agriculture, 1989). It may have been because of the pathogenesis of diabetes in animal models is most likely similar to the pathogenesis in humans. Streptozotocin (STZ) has been extensively used to induce diabetes for various diabetes studies in laboratory animals. STZ has been reported to be capable of generating reactive oxygen species resulting in oxidative stress and cell death. Also, STZ has been found to be a better chemical inducer for diabetes than alloxan (Szkudelski, 2001).

The present study was designed to demonstrate the histological changes on the endocrine component of pancreas in STZ induced diabetes in a rat model.

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MATERIALS AND METHODS

Care and management of experimental animals

The present study was carried on approval from Institutional Animal Ethics Committee of Veterinary College, Bangalore. Adult male Albino Wistar rats weighing 220±40g were maintained under standard laboratory conditions. The animals were randomly separated into two groups, group I as normal control, while group II as diabetic.

Induction of experimental diabetes

After a two-week acclimatization period, DM was induced experimentally in group II by a single intraperitoneal injection of a freshly prepared STZ solution (Sigma Chemicals, USA) dissolved in 0.1 M ice-cold citrate buffer (pH 4.5) at a dose of 45 mg/kg to overnight-fasted rats (Babu and Prince, 2004). Control rats received an intraperitoneal injection of citrate buffer alone. After three days of STZ administration, blood glucose levels of each rat were determined. Rats with a blood glucose level above 300mg/dL were considered diabetic and included in this study. The blood glucose level was measured using commercially available biochemical kits (Span Diagnostics, Bangalore) (Tietz, 1976).

Histological Evaluation

Two animals from each group were euthanized on 15th, 30th and 45th day of the experiment using an overdose of ether for studying the progressive effects of STZ. Animals were immediately dissected and pancreas was observed for evidence of gross pathology. For the light microscopic examinations, samples from tail portions of the pancreas were fixed in 10 percent neutral buffered formalin and embedded in paraffin. The paraffin blocks were cut into pieces with a thickness of 4 μm. The sections were stained with Hematoxylin and Eosin (H&E) (Bancroft and Gamble, 2008) for the evaluation of islets injury.

Statistical Analysis

Data were analyzed using a commercially available statistics software package (Graph Pad Prism for Windows Version 5.0). All values are presented as Mean ± Standard Error (SE). The data were analyzed using paired sample student t test ($p < 0.001$; two tailed).

RESULTS AND DISCUSSION

A significant ($p < 0.001$) decrease in the body weight was observed in the diabetic group when compared to the control. The blood glucose level was significantly increased ($p < 0.001$) in the diabetic group when compared to the control.

The diabetic control rats appeared grossly emaciated. The pancreas showed slight congestion and progressive decrease in the size which became appreciable from 15th day of the present study. On 45th day, the pancreas was atrophied and appeared as a thin gelatinous strip.

Histopathology of islets of Langerhan's of pancreas of control animals revealed normal architecture with compact arrangement of β- cells and non β- cells throughout the study. In the present study, post STZ injection, the pancreas showed numerous lobules with normal appearing exocrine component in diabetic control rats on 15th day. However, pancreatic islets were reduced in number per lobule and showed loss of normal architecture. The normal distribution of non

β-cells and β-cells was altered with much reduction in the number of β-cells and disorderly arranged non β- cells. The β-cells were swollen, vacuolated, necrotic or elongated and fusiform with condensed nucleus and showed loss of cytoplasmic granularity.

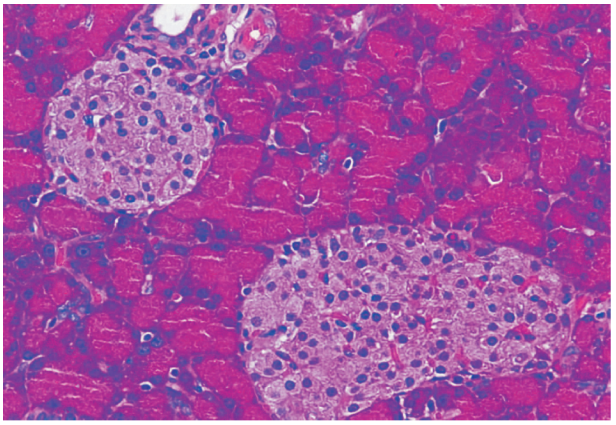

Fig 1A. Islet of control animal showing normal architecture with compact arrangement of β- cells and non β- cells on 15th day (H&E X 200)

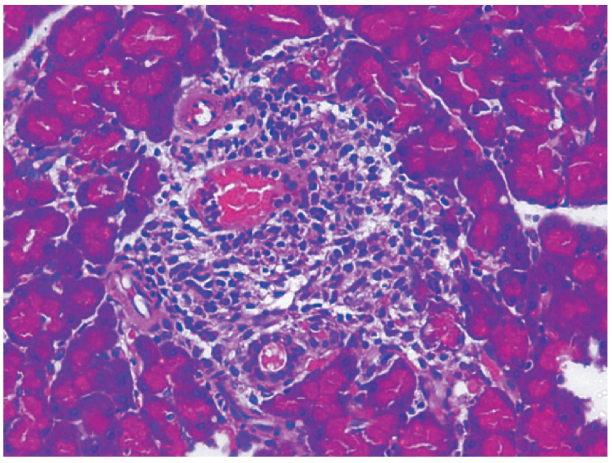

Fig 1B. Islet of diabetic control showing atrophied and vacuolated β- cells on 15th day (H&E X 200)

On 30th day post STZ injection, there was further decrease in the number of islets which appeared shrunken and showed increased severity in those lesions that appeared on 15th day in β-cells. On 45th day post STZ injection, occasional small sized islets were appreciated which were difficult to locate. There was severe reduction in the number of β-cells. Occasional persisting β-cells were highly swollen, vacuolated and showed loss of cytoplasmic granularity. This was in agreement with earlier reports. (Papaccio *et al.,* 2000 and Mir *et al.,* 2008). They appeared irregular in shape, reduced in the size, with some assuming 'star fish' appearance. Besides, there was an increase in the number of non β- cells and mild degrees of fibrosis with infiltration of a few inflammatory cells were also observed. The histopathology of islets in diabetic rats indicated progressive destruction of β-cells from 15th to 45th day of the investigation.

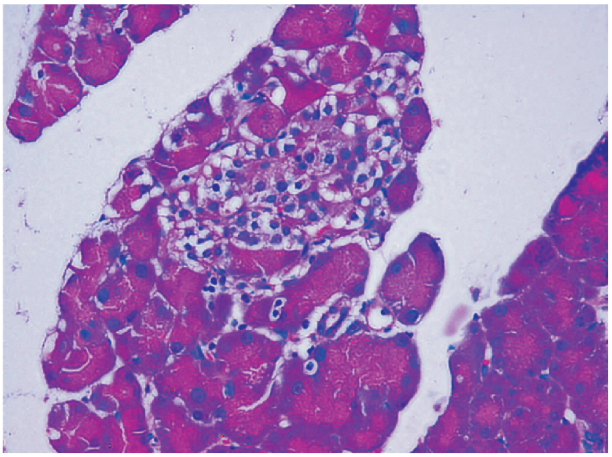

Fig 1C. Islet showing extensive vacuolation and necrosis of β- cells on 30th day (H&E X 200)

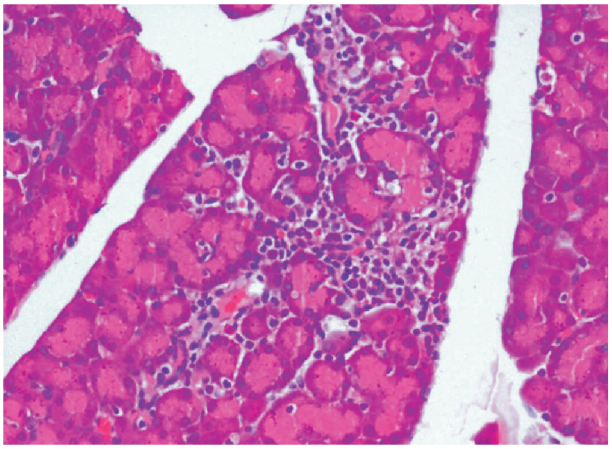

Fig 1D. Islet showing atrophied and "starfish" appearance on 45th day (H&E X 200)

According to the American Diabetes Association (ADA), most common symptoms of diabetes include polydipsia, weight loss and polyphagia those were evidently present in the diabetic groups in the present experiment. The elevation of blood sugar level on 3rd day confirmed the establishment of diabetes mellitus in rats which is attributed to the selective cytotoxicity of STZ on β- cells (Bedoya *et.al.,* 1996).

The decrease in cellularity within islets observed in the study reflects the cytotoxity of STZ. The reduction in the number of β- cells was also noticed. STZ possesses diabetogenic effect mediated through pancreatic β-cell destruction. STZ appears to cause cytotoxicity by a number of mechanisms. STZ on entry into the β- cells via a glucose transporter (GLUT2) gets spontaneously decomposed to form an isocyanate compound and a methyldiazohydroxide which cause intra molecular carboxylation and alkylation of cellular components respectively especially that of DNA of β-cells (Varva, 1960). However, the DNA damage of β-cells of pancreas is mainly by alkylation with carbonium ion produced by methyldiazohydroxide (Elsner *et.al.,* 2000).

The DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of STZ than just DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies a substrate for xanthine oxidase resulting in the formation of super oxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are generated. Further, STZ liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, β-cells undergo destruction by necrosis. STZ selectively destroys the pancreatic insulin secreting β- cells, leaving less active cells and result in a diabetic state (Szkudelski, 2001).

In the present study, β- cells in some islets were found to be fusiform. The change in the shape of cells can be attributed to the partial damage of STZ to some β- cells. Aybar *et.al.* (2001) have reported that use of lower dose of STZ produced an incomplete destruction of pancreatic β-cells even though rats became permanently diabetic.

Brownlee (2001) postulated a concept that link hyperglycaemia-induced damage by different mechanisms that finally leads to cellular stress**.** Firstly, hyperglycemia increase movement of glucose through polyol pathway and sorbitols are produced which in turn causes osmotic stress to cells and dihydronicotine amide adenine dinuleotide phosphate (NADPH) is consumed, depleting intracellular glutathione. Secondly, hyperglycemia increases concentrations of advanced glycation end products. These glycosylated proteins are formed by non-enzymatic reactions and changes in protein structure may alter their cellular functions. Thirdly, glucose activates various isomers of protein kinase C which in turn affects the expression of nitric oxide, endothelin, nuclear factor kappa B (NF-kB) and plasminogen activator inhibitor. Finally, hyperglycemia increases the flux of glucose through the hexasomine pathway effecting inflammatory mediators and insulin resistance. The combined effect of these mechanisms results in over-production of superoxides by the mitochondrial electron-transport chain, causing cellular stress and damage that was clearly appreciated in the form of islets injury in the present study. STZ induced hyperglycaemia has

been described as a useful experimental model to study the activity of hypoglycaemic agents. So the result of the present study is useful to evaluate the hypoglycaemic agents in pre-clinical trials.

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