

Quantitative Studies of Hepatocytes in Periportal and Perivenous Zones of the Liver Lobules in Diabetic Rats

Mohammad Jafar Golalipour and Soraya Ghafari
Department of Histology and Embryology,
Gorgan University of Medical Sciences, Gorgan, Iran

Abstract: This study was designed to evaluate the morphometric changes of parenchymal cells in the periportal (Z1) and perivenous (Z3) zones of the liver lobules that occur after Streptozotocin-induced diabetes in rats. Twenty male rats were allocated in two groups of normal and diabetic. Hyperglycemia in rats induced by 80 mg kg⁻¹ Streptozotocin intraperitoneally. The blood glucose concentration was measured by using a Glucometer in 1st, 3rd and 5th week. After five weeks, with using anesthesia liver removed. Liver specimens were fixed in buffered formaldehyde and embedded in paraffin. Hematoxylin and eosin stained sections were used for quantitative morphometric analysis. Morphometric parameters in mononuclear cells were measured by Olysia software. All data are shown as mean with standard error of means and analyzed using the Student's t-test and p-value less of 0.05. Mean areas of hepatocyte, their nuclei and nucleolus were reduced by approximately 1.29, 7.27 and 0.76% in zone 1 in the diabetic rats in comparison with the control group. While mean areas of hepatocyte, their nuclei and nucleolus were increased by approximately 5.26, 2.53 and 3.10%, respectively, in zone 3 in the diabetic and control rats. It is concluded that Streptozotocin injection leads to reduction in area of hepatocytes and their nuclei and nucleolar in zone 1 and an increase in area of hepatocytes and their nuclei and nucleolar in zone 3.

Key words: Morphometry, hepatocyte, diabetes, liver, Streptozotocin, rat

INTRODUCTION

Diabetes is a chronic disease with a relatively high prevalence in many populations throughout world (Jerrold, 2000). The liver plays a central and crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for the maintenance of blood glucose levels and of a continued supply to organs that require a glucose energy source. The liver uses glucose as a fuel and also has the ability to store it as glycogen and synthesize it from noncarbohydrate precursors (gluconeogenesis) (Levinthal and Tavill, 1999).

This central role for the liver in glucose homeostasis offers a clue to the pathogenesis of glucose intolerance in liver diseases but little insight into the mechanisms of liver disease in diabetes mellitus (Levinthal and Tavill, 1999). Liver disease may cause or contribute to, be coincident with, or occur as a result of diabetes mellitus such as: hepatitis, autoimmune biliary disease, glycogen deposition, fibrosis and cirrhosis (Silverman *et al.*, 1990; Levinthal and Tavill, 1999). Quantitative morphometric changes of the liver are largely unexplored in diabetes.

Corresponding Author: Mohammad Jafar Golalipour, Department of Histology and Embryology,
Gorgan University of Medical Sciences, P.O. Box 49175-553, Gorgan, Iran
Tel/Fax: +98(171)4425165, 4425660

Streptozotocin (STZ), a N-nitroso-N-methylurea derivative of 2-deoxy-D-glucose, is a diabetogenic agent acting through the selective destruction of pancreatic islet β -cells (Junod *et al.*, 1967; Stauffacher *et al.*, 1970) and widely used for induction of diabetes in experimental animals (Aughsteeen *et al.*, 2006; Koyuturk *et al.*, 2005; Bolkent *et al.*, 2004; Stauffacher *et al.*, 1970). It is known that STZ displays hepatotoxic and nephrotoxic activity (Bolkent *et al.*, 2004; Piyachaturawat *et al.*, 1991).

This study was aimed at exploring the quantitative morphometric changes of parenchymal cells in the periportal (Z1) and perivenous (Z3) zones of the liver lobules that occur at early stages in Streptozotocin-diabetic rats using quantitative morphometry under a light microscope.

MATERIALS AND METHODS

Animals

Twenty male adult Wistar albino rats with 125-175 g weight were used for this study. The rats were housed in groups of three in standard animal cages and kept under standard Laboratory conditions in Gorgan University of Medical Sciences in 2006. Animals had free access to rat pelleted chow and tap water.

Experimental Design

Twenty rats were allocated in the following groups.

Group I : Normal control rats. Saline IP for one time.

Group II : Diabetic rats. 80 mg kg⁻¹ STZ IP one dose.

In this group, hyperglycemia was induced with the single intraperitoneal (i.p.) injection of Streptozotocin, purchased from Sigma, 80 mg kg⁻¹ body weight dissolved in distilled water just before use to overnight fast rats. Glucose levels from the blood of the tail vein of the rats were measured with a Glucometer.

Glucose Tolerance Test

Intraperitoneal Glucose Tolerance Test (GTT) was performed on 16 h fasted rats using 2 g glucose kg⁻¹ body weight. In all groups, blood was collected from the animals by tail snipping at 0, 30, 90 and 120 min after glucose load. Also glucose test were performed after IP injection STZ in 1, 3 and 5 weeks.

All the animals were deeply anesthetized with ether after 5 weeks from the beginning of the experiment. Then the livers were removed and fixed in natural buffered formaldehyde fixative. Section of hepatic tissue obtained from the left lateral and right posterior lobes and sliced at 5 mm thickness and embedded in paraffin wax after overnight machine processing for histological examination. Hematoxyline and eosin (H and E)-stained sections (Bancroft and Gamble, 1990) at 5 μ m thickness were used for morphological and morphometric analysis.

Morphometric Measurements

The area measurements of hepatocytes were made using the Olympus BX-51T-32E01 research microscope connected to DP 12 Camera with 3.34 million pixel resolution and Olysia Bio software (from: Olympus Optical Co. Ltd., Tokyo-Japan) in zone 1: liver cells near the portal area (Z1: periportal) and zone 3: hepatocytes in the centrilobular regions of the hepatic lobule (Z3: perivenous).

For each hepatocyte, total cellular area, nuclear area and nucleolar area were measured. The outline of each hepatocyte initially was measured followed by the outline of the nucleus using a 40X objective. A separate measurement for nuclei and nucleoli was performed using the same methodology but using a 100X oil objective. At least 50 nuclei from each zone (total 100) were measured from each liver.

Morphometric Variables

The following variables were used in this study to measure the light microscopy changes (Zaitoun *et al.*, 2005):

Area	: The total area of the whole hepatocyte, nucleus and nucleolus in mononuclear cells.
Perimeter	: The perimeter of whole hepatocytes.
Diameter	: The diameter of nucleus.
Long axis	: The long axis of the nucleus.
Short axis	: The short axis of the nucleus.
Hepatocyte area ratio	: The ratio of mean area of whole hepatocytes to the mean area of their nuclei.
Nuclear area ratio	: The ratio of the mean area of nuclei to the mean area of their nucleoli.
Cytoplasmic area	: The hepatocyte area minus nuclear area
Area difference	: Nuclear area minus nucleolar area.

Statistical Analysis

Results were expressed as mean and standard error of the means (SE). The statistical analysis was performed with the Student's t-test by means of the SPSS Version 11.5 software two-tailed p-values less than 0.05 were considered to indicate statistical significance.

RESULTS

Changes in the Total Area of Hepatocytes

Quantitative parameters of hepatocyte area, perimeter, cytoplasmic area and cell/nuclear ratio in hepatocytes of zone 1 and zone 3 in diabetic and normal rats are given in Table 1.

The mean area of hepatocyte in Z1 was smaller in diabetic group in comparison with control group. This difference was not significant ($p < 0.05$). Whereas in zone 3 the mean area of hepatocytes in diabetic group was greater than that in control group, significantly ($p < 0.05$).

Changes in the Nuclei

The changes in the nuclei in both groups of animals are shown in Table 2. Nuclear area and perimeter, nuclear diameter, long axis and short axis were significantly reduced in diabetic group in Z1. The mean area of hepatocyte nuclear in Z1 in diabetic group was smaller than that in control group. This difference was significant ($p < 0.05$). The mean area of hepatocyte nuclear in Z3 had a rare increase in diabetic group in comparison with control group, which was not found statistically significant.

Changes in the Nucleolar Area

Table 3 shows the results of nucleolar areas. The changes in nucleolar areas in both groups were not significant in Z1 and Z3. Furthermore there were significant changes for Z1 in area difference and nuclear/nucleolar ratio between diabetic and control group.

Table 1: Quantitative parameters of hepatocyte area, perimeter, cytoplasmic area and cell/nuclear ratio in hepatocytes of zone 1 and zone 3 in diabetic and normal rats (n=10)

Groups	Hepatocyte area (μm^2)		Hepatocyte perimeter (μm)		Cytoplasmic area (μm^2)		Cell/nuclear ratio	
	Z1	Z3	Z1	Z3	Z1	Z3	Z1	Z3
I Control	260.82±4.92	263.90±4.80*	67.24±0.67	68.09±0.64	212.05±4.58	216.11±4.50*	5.46±0.10*	5.66±0.11
II STZ	257.45±4.08	277.77±3.45*	65.68±0.51	68.02±0.48	212.24±3.54	228.78±3.19*	5.85±0.09*	5.81±0.09

Results are expressed as mean±SE of the mean. *: p<0.05

Table 2: quantitative parameters of hepatocyte nuclei in zone 1 and zone 3 in diabetic and normal rats (n=10)

Groups	Nucleus area (μm^2)		Nucleus perimeter (μm)		Diameter of nucleus	
	Z1	Z3	Z1	Z3	Z1	Z3
I Control	48.76±0.81*	47.78±0.80	29.50±0.26**	28.89±0.26	7.75±0.04**	7.56±0.06
II STZ	45.21±0.85*	48.99±0.74	27.59±0.33**	28.24±0.26	7.45±0.06**	7.47±0.06

Groups	Long axis (μm)		Short axis (μm)	
	Z1	Z3	Z1	Z3
I Control	8.15±0.05**	7.96±0.07	7.35±0.05*	7.17±0.07
II STZ	7.78±0.07**	7.83±0.07	7.13±0.07*	7.12±0.06

Results are expressed as mean±SE of the mean. *: p<0.05, **: p<0.001

Table 3: Quantitative parameters of nucleolar areas, area difference (Nuclear minus nucleolar areas) and nuclear/nucleolar ratio from hepatocytes in zone 1 and 3 in diabetic and normal rats (n=10)

Groups	Nucleolus area (μm^2)		Area difference (μm^2)		Nuclear/Nucleolar ratio	
	Z1	Z3	Z1	Z3	Z1	Z3
I Control	5.25±0.12	5.16±0.13	43.51±0.77**	42.62±0.74	9.85±0.22*	9.87±0.21
II STZ	5.21±0.14	5.32±0.13	39.99±0.77**	43.66±0.69	9.25±0.20*	9.80±0.22

Results are expressed as mean±SE of the mean. *: p<0.05, **: p<0.001

DISCUSSION

In the present study, we assessed the histological changes at the light microscopy level using quantitative techniques and we have studied the effect of STZ on zone 1 and 3 of the liver lobules. The morphometric analysis determined that the area of hepatocytes decreased in zone 1 that was not significant whereas in zone 3, area of hepatocytes increased significantly in comparison with control group.

The functions of STZ on the liver were explained by the different mechanisms. Single dose administration of STZ produces diabetogenic effects in rats (Koyuturk *et al.*, 2005; Bolkent *et al.*, 2004; Stauffacher *et al.*, 1970). Also, STZ exhibits hepatotoxic activity on the liver in rats (Piyachaturawat *et al.*, 1991; Watkins *et al.*, 2000; Bolkent *et al.*, 2004). The STZ diabetic animals may exhibit most of the diabetic complications through oxidative stress (Petlevski *et al.*, 2003; Ozturk *et al.*, 1996). Oxidative stress investigates by a decrease in the activity of antioxidant enzymes (Rajasekaran *et al.*, 2005; Bulkent *et al.*, 2004; Rauscher *et al.*, 2000; Gunes *et al.*, 1999) and an increase in the lipid peroxidation and free radicals. The efficiency of this defense mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage (Wohaieb and Godin, 1987). In diabetes mellitus excess glycogen accumulation by gluconeogenesis (Consoli *et al.*, 1989; Ferrannini *et al.*, 1990) leads to vacuolization in cytoplasm and hepatocyte nuclei (Glick *et al.*, 1987) and may exhibit hepatomegaly and liver abnormalities (Chatila and West, 1996; Sanchez *et al.*, 2000). Oxidative energy metabolism and gluconeogenesis protective metabolism are catalyzed mainly in the periportal zone, glucose utilization, glycogen storage and lipid formations are predominant in the perivenous zone (Jungermann and Kietzman, 1996;

Katz, 1992). The defense intracellular mechanisms and the activity of nuclei and subcellular structures during mechanism pathways in hepatocytes may be effect on the measurement of cells and cause the morphometric changes in the liver in diabetic rats. Increasing of hepatocytes size is comparable with studies of other authors (Kume *et al.*, 1994, 2005; Sanchez *et al.*, 2000). Herrman *et al.* (1999) reported diabetes mellitus in both humans and animals leads to structural and functional changes including hepatomegaly. Also Kume *et al.* (1994), observed an appearance of cytomegalic hepatocytes in some diabetic mice, due to an increase in number of intracytoplasmic acidophilic granules and mitochondria. However some studies have shown enhance reduction of hepatocytes volume in both of zonation in STZ-diabetic rats (Noorafshan *et al.*, 2005).

We also found a reduction in the hepatocyte nuclear area in zone 1 significantly and a rare increase of the hepatocyte nuclear area in zone 3 and a small change in area of their nucleolar that were not significant after STZ injection. Nuclei are responsible for production of RNA and consequent protein synthesis. In non-mitotic cells, nuclear volume had been shown to be closely related to DNA content and the level of activity (Sorensen *et al.*, 1990; Christie and Le Page, 1961; Henrique *et al.*, 1997). Even in mitotic cells such as hepatocytes of rat, changes in nuclear size without change in the ploidy have been related to changes in protein synthesis and nuclear activity (Kume *et al.*, 1994; Christie and Le Page, 1961). Loss of rough endoplasmic reticulum, reduced amino-acid and a decrease in reticulum-bound ribosomes were occurred in insulin-deficient animals (Thompson *et al.*, 1981; Lenk *et al.*, 1992; Kume *et al.*, 1994; Thakran *et al.*, 2004). These findings suggest that patients with diabetes have a defect in secretory protein synthesis. Therefore, decreasing in nuclear or cell volume may relate a reduction of metabolic activity of liver cells and inadequate levels of insulin (Noorafshan *et al.*, 2005). Although, Doi *et al.* (1997) had been shown the area of hepatocyte nuclei in diabetic mice was about two times larger than that in control mice. Also, they reported that the nuclei of binuclear hepatocytes of control mice were round and identical in ultrastructural appearance.

In this study other parameters such as cytoplasmic area, cell/nuclear ratio, diameter of nuclei, area difference and nuclear/nucleolar ratio have changed. The consideration of the data established these changes were in agreement with the changes of hepatocytes and their nuclei area.

In conclusion, streptozotocin injection lead to a reduction in area of hepatocytes and their nuclei in zone 1 and an increase in area of hepatocytes and their nuclei in zone 3 of the liver lobules at early stages. The investigation of these changes may be useful for studies of liver pathology in patients with diabetes mellitus.

ACKNOWLEDGMENT

We thanks from research department of Gorgan University of Medical Sciences for financial support.

REFERENCES

- Aughsteeen, A.A., M.B. Al-Khair and A.A. Suleiman, 2006. Quantitative morphometric study of the skeletal muscles of normal and Streptozotocin-diabetic rats. *JOP. J. Pancreas*, 7: 382-389.
- Bancroft, J.D. and M. Gamble, 1990. *Theory and Practice of Histological Techniques*. 3rd Edn., London Churchill Livingstone, pp: 109-112.
- Bolkent, S., R. Yanardag, O. Karabulut-Bulan and O. Ozsoy-Sacan, 2004. The morphological and biochemical effects of glibornuride on rat liver in experimental diabetes. *Hum. Exp. Toxicol.*, 23: 257-264.
- Chatila, R. and A.B. West, 1996. Hepatomegaly and abnormal liver tests due to glycogenesis in adults with diabetes. *Med. Balt.*, 75: 327-333.

- Christie, G.S. and R.N. Le Page, 1961. Enlargement of liver cell nuclei: Effect of dimethylnitrosamine on size and deoxyribonucleic acid content. *Lab Invest.*, 10: 729-743.
- Consoli, A., N. Nurjhan, F. Capani and J. Gerich, 1989. Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes*, 38: 550-557.
- Doi, K., J. Yamanouchi, E. Kume and A. Yasoshima, 1997. Morphologic changes in hepatocyte nuclei of streptozotocin (SZ)-induced diabetic mice. *Exp. Toxicol. Pathol.*, 49: 295-299.
- Ferrannini, E., A. Lanfranchi, F. Rohner-Jeanraud, G. Manfredini and G. VandeWerve, 1990. Influence of long-term diabetes on liver glycogen metabolism in the rat. *Metabolism*, 39: 1082-1088.
- Glick, M.E., J.C. Hoefs and H. Meshkinpour, 1987. Glucose intolerance and hepatic, biliary tract and pancreatic dysfunction. *Dig. Dis.*, 5: 78-96.
- Gunes, H.V., I. Degirmenci, M. Aydın, B. Bozan, E. Aral, Z. Tunalier, C. Ustuner, M. Ercakar, K.H.C. Baser and A. Basaran, 1999. The effects of *Rumex patientia* L. and *Urtica dioica* L. on some blood and urine parameters and liver and kidney histology in diabetic rats. *Turk. J. Med. Sci.*, 29: 227-232.
- Henrique, R.M.F., R.A.F. Monteiro, E. Rocha and M.M. Mrini-Abreu, 1997. A stereological study on the nuclear volume of cerebellar granular cells in aging rats. *Neurobiol. Aging*, 18: 199-203.
- Herrman, C.E., R.A. Sanders, J.E. Klaunig, L.R. Schwarz and J.B. Watkins, 1999. Decreased apoptosis as a mechanism for hepatomegaly in streptozotocin-induced diabetic rats. *Toxicol. Sci.*, 50: 146-151.
- Jerrold, M.O., 2000. Diabetes Mellitus. In: *Cecil Text Book of Medicine*. WB Saunders, pp: 1292-1293.
- Jungermann, K. and T. Kietzmann, 1996. Zonation of parenchymal and nonparenchymal metabolism in liver. *Annu. Rev. Nutr.*, 16: 179-203.
- Junod, A., A.E. Lambert, L. Orci, R. Pictet, A.E. Gonet and A.E. Renold, 1967. Studies of the diabetogenic action of streptozotocin. *Proc. Soc. Exp. Biol. Med.*, 126: 201-205.
- Katz, N.R., 1992. Metabolic heterogeneity of hepatocytes across the liver acinus. *J. Nutr.*, 122: 843-849.
- Koyuturk, M., S. Tunalı, S. Bolkent and R. Yanardag, 2005. Effects of vanadyl sulfate on liver of streptozotocin-induced diabetic rats. *Biol. Trace Elem. Res.*, 104: 233-247.
- Kume, E., Y. Ohmachi, S. Itagaki, K. Tamura and K. Doi, 1994. Hepatic changes of mice in subacute phase of streptozotocin (SZ)-induced diabetes. *Exp. Toxicol. Pathol.*, 46: 368-374.
- Kume, E., C. Aruga, K. Takahashi, S. Miwa, E. Dekura, M. Itoh, Y. Ishizuka, H. Fujimura, W. Toriumi and K. Doi, 2005. Morphological and gene expression analysis in mouse primary cultured hepatocytes exposed to streptozotocin. *Exp. Toxicol. Pathol.*, 56: 245-253.
- Lenk, S.E., D. Bhat, W. Blakeney and W.A. Jr. Dunn, 1992. Effects of streptozotocin-induced diabetes on rough endoplasmic reticulum and lysosomes of rat liver. *Am. J. Physiol.*, 263: 856-862.
- Levinthal, G.N. and A.S. Tavill, 1999. Liver disease and diabetes mellitus. *Clin. Diabetes*, 17: 2.
- Noorafshan, A., B. Esmail-Zadeh, S. Bahmanpour and A. Poost-Pasand, 2005. Early stereological changes in liver of Sprague-Dawley rats after streptozotocin injection. *Indian J. Gastroenterol.*, 24: 104-107.
- Ozturk, Y., V.M. Altan and N. Yildizoglu-Ari, 1996. Effects of experimental diabetes and insulin on smooth muscle functions. *Pharmacol. Rev.*, 48: 69-112.
- Petlevski, R., M. Hadzija, M. Slijepcevic, D. Juretic and J. Petrik, 2003. Glutathione S-transferases and malondialdehyde in the liver of NOD mice on short-term treatment with plant mixture extract P-9801091. *Phytother. Res.*, 17: 311-314.
- Piyachaturawat, P., J. Poprasit and T. Glinsukon, 1991. Gastric mucosal secretions and lesions by different doses of streptozotocin in rats. *Toxicol. Lett.*, 55: 21-29.

- Rajasekaran, S., N. Sriram, P. Arulselvan and S. Subramanian, 2005. Effect of Aloe vera leaf gel extract on membrane bound phosphatases and lysosomal hydrolases in rats with streptozotocin diabetes. *Pharmazie*, 62: 221-225.
- Rauscher, F.M., R.A. Sanders and J.B. Watkins, 2000. Effects of piperine on antioxidant pathways in tissues from normal and streptozotocin-induced diabetic rats. *J. Biochem. Mol. Toxicol.*, 14: 329-334.
- Sanchez, S.S., A.V. Abregu, M.J. Aybar and A.N. Sanchez Riera, 2000. Changes in liver gangliosides in streptozotocin-induced diabetic rats. *Cell Biol. Int.*, 24: 897-904.
- Silverman, J.F., K.F. Obrien, S. Long, N. Leggett, P.G. Khazanie and W.J. Pories, 1990. Liver pathology in morbidly obese patients with and without diabetes. *Am. J. Gastroenterol.*, 85: 1349-1355.
- Sorensen, F.B., I.B. Kristensen, F. Grymer and A. Jakobsen, 1990. DNA-index and stereological estimation of nuclear volume in primary and metastatic malignant melanomas: A comparative study with analysis of heterogeneity. *APMIS.*, 98: 61-70.
- Stauffacher, W., I. Burr, A. Gutzeit, D. Beaven, J. Veleminsky and A.E. Renold, 1970. Streptozotocin diabetes: Time course of irreversible B-cell damage; further observations on prevention by nicotinamide. *Proc. Soc. Exp. Biol. Med.*, 133: 194-200.
- Thakran, S., M.R. Siddiqui and N.Z. Baquer, 2004. *Trigonella foenum graecum* seed powder protects against histopathological abnormalities in tissues of diabetic rats. *Mol. Cell Biochem.*, 266: 151-159.
- Thompson, E.W., W.C. Parks and R.L. Drake, 1981. Rapid alterations induced by insulin in hepatocyte ultrastructure and glycogen levels. *Am. J. Anat.*, 160: 449-460.
- Watkins, J.B., P.A. Gardner, J.D. Feczko and K.M. Klueber, 2000. Streptozotocin and insulin-dependent diabetes induce changes in hepatic cytoarchitecture in mice. *Int. J. Toxicol.*, 17: 401-405.
- Wohaieb, S.A. and D.V. Godin, 1987. Alterations in free radical tissue-defense mechanisms in Streptozotocin-induced diabetes in rat. Effects of insulin treatment. *Diabetes*, 36: 1014-1018.
- Zaitoun, A.A., G. Apelqvist, H. Al-Mardini, T. Gray, F. Bengtsson and C.O. Record, 2005. Quantitative studies of liver atrophy after portacaval shunt in the rat. *J. Surg. Res.*, 131: 225-232.