A STEREOLOGICAL STUDY OF DIABETIC KIDNEY FOLLOWING ADMINISTRATION OF DIFFERENT DOSES OF STREPTOZOTOCIN

[*]ZAHRA HEIDARI, [*]HAMID-REZA MAHMOUDZADEH SAGHEB, [**]HOOSHANG RAFIGHDOOST, [**]ABBAS-ALI MOEIN, [***]MOHAMMAD-HOSEIN NOORI MUGAHI, [***]BAGHER MINAII

[*]Department of Histology, [**]Department of Anatomy, Faculty of Medicine, Zahedan University of Medical Sciences, IRAN. [***]Department of Histology, Faculty of Medicine, Tehran University of Medical Sciences, IRAN

ABSTRACT

The absolute glomerular volume and total glomerular number as well as renal volume in diabetic rats following administration of different doses of Stereptozotocin (STZ) have been estimated by using unbiased stereological methods. 30 male Wistar rats were randomly divided into 5 groups. Diabetes were induced by IP injection of 15mg/kg, 45mg/kg and 90 mg/kg of STZ. Fifty six days after IP injection, animals were anesthetized by ketamine hydrochloride, the kidneys were excised and fixed in modified Lilli´s solution. After tissue processing and staining, stereological methods used to estimate volume of different zones of kidney, absolute glomerular volume and total glomerular number. This investigation revealed that increment of cortical tissue volume in all groups and increase in medullary volume only in the group 90 mg/kg of STZ (p<0.01) were administered. Glomerular volume was increased significantly in all groups (p<0.01) but no changes in total glomerular number were detected (p>0.05). Animal weight also showed significant changes in 45 mg and 90 mg groups in comparison with the control group (p<0.01). This study showed that at least administration of more STZ in short period might have more effect and causes more imbalances in cells turnover. The results of this study were in accordance with other qualitative and quantitative surveys. Moreover, it was suggested that in the early stages of the induced diabetes mellitus by STZ, the inducer has different effects and more doses may cause more induction and effects on kidneys.

Key words: Diabetes, Streptozotocin, Kidney, Glomeruli, Stereology

INTRODUCTION

Renal hypertrophy and hyperfiltration are inherent features in the course of experimental and human diabetes mellitus (1-3). The degree of renal enlargement and glomerular hyper-filtration is thought to be a predictor for development of the late diabetic nephropathy (1,2). Kidney growth has been found to correlate with the degree of hyperglycemia and to proceeds with increase in glomerular filtration rate. A variety of clinical and experimental studies have been conducted in order to characterize the early diabetic renal changes that will be followed by increase in the urinary albumin excretion and kidney lesions. Several metabolic, functional and structural changes in alloxan and Streptozotocin (STZ) diabetic rats have fundamental similarities with those in diabetic patients (1,4-8). There are several techniques including chemical destruction, surgical removal of the beta cell mass or even pancreatectomy for induction of experimental diabetes (8-11). Different chemical agents are capable to produce alterations related to the diabetic condition of which two of the most widely used are alloxan and streptozotocin (STZ) (8-13). Streptozotocin (STZ) is a nitrosourea derivative isolated from *Streptomyces griseus*. It can induce severe insulin-deficient diabetes in rats and other rodents when given as a single large dose (50-100mg/kg in rats) or as multiple smaller doses. In the latter case, diabetes develops more gradually and appears to have an autoimmune, rather than a toxic basis (9). Diabetes induced by a single intravenous or intraperitoneal injection of STZ is properly the most widely used experimental model for insulin-dependent diabetes mellitus. By the use of a dosage of 50-60mg/kg, insulin levels typically fall to 10-30% of normal, leading to hyperglycemia (20-30 mmol/l), polyuria, polydipsia and weight loss. However, severe ketosis does not develop and animals can survive for some weeks without

Correspondence:.Zahra. Heidari, Department of Histology, Faculty of Medicine, Zahedan University of Medical Sciences, Iran. E-Mail: histology_iri@hotmail.com

insulin replacement. Higher dosages induce more profound insulin deficiency and results in spontaneous ketosis and death if insulin is not administrated (9). STZ causes beta-cell necrosis and diabetes supervenes within 1-2 days through damages to the beta-cell membrane, and induction of DNA strand breaks which leads to activation of poly (ADP-ribose) synthetase and NAD depletion (14). Due to relations between structure and function, the study of glomerular volume is important for understanding of initiation and progression of diabetic glomerulopathy (15). In other investigations, changes in the renal volume and absolute glomerular volume in diabetic patients and rats have been observed (15,16). Changes in glomerular number following diabetes and other renal diseases have also been reported (17 – 19). The aim of the present study was to estimate renal volume, absolute glomerular volume and total glomerular number in diabetic rats following administration of different doses of STZ by the use of the unbiased stereological methods.

MATERIALS AND METHODS

30 male Wistar rats with an average weight of 110±5 gr. were randomly divided into 5 groups. Each group had 6 animals. There was a constant cycle of 12 h light and 12 h darkness and temperature was maintained at 19-22 °C. Animal had access to food and water *ad libitum*. Diabetes was induced by an IP injection of 15 mg/kg, 45mg/kg and 90 mg/kg of Sterepto-zotocin in 1.0 ml of acetate buffer in three groups. The control rats were treated with the same volume of distilled water and the sham control rats were injected intrapentoneally 1.0 ml of acetate buffer. Fifty-six days after IP injection, animals were anesthetized by ketamine hydrochloride. Following laparotomy kidneys were fixed in modified Lilli´s solution by arterial perfusion. Kidneys were excised, decapsulated and immersed in the same fixative for 48 hours at room temperature. The fixed kidneys were embedded in 7% Agar and they were cut by means of a tissue slicer (20,21). The slices were arranged in a number of sequence on meshed tissue processing baskets and then they were processed and embedded in paraplast. At each level, three sections were cut to a thickness of 7 micrometer by a microtome and were stained with H&E. Stereological study was performed using light microprojectors (Ken-A-Vision X-1000-1, U.S.A) and the image was projected on a table. A fine grid of points was

superposed. Point counting using Cavalieri principle was used to estimate the volume of cortex, medulla and whole kidney. The first section from each triple was chosen and then each kidney was wholly analyzed at a final magnification of î 32 and volume was estimated from the following equation (15,16, 20,22,23)

$$V = \frac{\sum_{i=1}^{m} P.a / p.\bar{t}}{M^2}$$

The volume fraction (V_v) of the components in the reference space (glomeruli) was obtained by point counting of the random cross sections by the use of the equation $V_v = S$ P (glomeruli)/ S P (cortex). Absolute volume of glomeruli was estimated indirectly by multiplying volume fraction by volume of the reference space (20,21). Physical disector was used to estimate the numerical density of glomeruli. The first and third sections from each slice were chosen as reference and look-up sections (23) and each pair of sections were mounted in the double microprojector and registered. Glomerular profiles were therefore compared with two parallel sections of the kidney. The integral test system comprises a series of frame with certain areas of forbidden and acceptance lines. Counting of the glomerular profiles was performed in the first (reference) but not in the second (look-up) section and they weren't cut by forbidden line either. In each kidney, about 30 to 60 frames of all sections were chosen and glomerular profiles were counted. Numerical density was estimated by the use of the equation

$$N_v = \frac{\sum Q}{a/f.h. \sum P}$$

By estimation of the numerical density of glomeruli and the volume of cortex the total number of glomeruli was obtained from the equation of N (glom) = V (cortex). N_V (glom, cortex) (17,18,22-24)

A One-Way ANOVA followed by an Scheffe test was used for analysis of differences between groups. In each case the null hypothesis was rejected if the probability of no differences was less than 5%.

RESULTS

At the end of the experiment, mean weight of animals significantly decreased in 45mg/kg and 90mg/kg diabetic groups compared with the control groups (p<0.01) but there were not

statistically significant differences between group which received 15mg/kg STZ in comparison with the control groups (p>0.05) (table 1).

Absolute volume of the cortical tissue increased 16%, 38% and 88% in groups which received 15mg/kg, 45 mg/kg and 90 mg/kg of STZ respectively, which is statistically significant when compared with control groups (p<0.01). Otherwise, enlargement of the medulla was only detectable in the group which were injected 90 mg/kg of STZ and it was estimated about 45% in comparison with control groups (p<0.01). The medullary volume of other two diabetic groups didn't show significant differences in comparison with control groups (P>0.05). However, estimation of the total renal volume of diabetic rats revealed an increment in comparison with control and sham control groups (p<0.01) that might be due to the increase in cortical tissue than medulla of the kidney (table 2). Absolute volume of glomeruli, a representative of the structural and functional units of kidney, increased 9.9% 27.4% and 60% in diabetic group that were administrated STZ at doses of 15mg/kg, 45mg/kg and 90 mg/kg respectively and in comparison with the control groups showed a significant increase in total volume of glomeruli (P<0.01).

On the other hand, estimation of the total number of glomeruli didn't show statistically significant changes between diabetic and normal kidneys (p>0.05). Although there was a decrease of about 2% in total glomerular number in the diabetic group, which were injected 90 mg/kg for induction of diabetes mellitus, the sham control group also showed the same decrease in the number of glomeruli (table 3).

DISCUSSION

Since diabetic cases that cause nephropathy pass different stages, quantitative data may be taken as an expression for the cause of disease. As it was supposed, different doses of STZ may cause different expression and modification of glomerular structure, therefore quantitative data may be important in terms of glomerular function in the course of diabetes. Basic to structural changes, alterations are taking place at the cellular level that can be stereologically determined at the structural level as a change in the volume or number of glomeruli. The hypertrophic changes have received much more

attention because of their possible role in development of the ultimate structural destruction (25). During the early stages, glomerular volume is enlarged 70 percent as compared with the glomeruli of non-diabetic subjects and the increment in glomerular volume is due to an increase in mesangial matrix as well as hypertrophy of other glomerular structure (26). As it has been proved, induction of diabetes mellitus by STZ causes increase in the glomerular volume and hypertrophy of kidney (15,27-29). This experiment showed that not only diabetes affects renal parenchyma but also different doses of STZ in a short period has different effects on the renal tissue and absolute glomerular volume. The most important outcome of the present study was that different single doses of STZ might cause different reactions in the kidney and administration of high doses of STZ results in more increase in glomerular volume which might be due to the increase in extracellular matrix. On the other hand, previous morphometric analyses of diabetic nephropathy have demonstrated increment of renal volume in human suffering from diabetes and laboratory animals which became diabetic by administration of STZ (27, 30-32). In accord with other reports in this study increase in total volume and especially cortical volume was estimated stereologically. However, there was an intimate dependency between the high dose of STZ and increment of renal volume. In contrast with other studies, results of this study revealed that increase in medullary volume was only significant in the group in which diabetes was induced by administration of STZ at the dose of 90 mg/kg. Contrary to other reports, which estimated lower number of renal glomeruli in long period of suffering by diabetes (31,32) in the present study, there were not significant changes in total glomerular number. It was also found that in the later stages of diabetes, exclusion of the occluded glomeruli results in a further increase in glomerular and mesangial volume, capillary length and surface area. These changes were attributed to compensatory hypertrophy due to the loss of nephrons (26). Although in our study no decrease in total nephron number was detected, hypertrophy of glomeruli and other cortical tissue was distinguished. The structural abnormality was related most closely to the changes to renal functional in diabetes which

Table 1. Mean±SEM of animal's weight (g) in diabetic groups compared with control and sham control groups. **P<0.01

Control	Sham Control	15 mg/kg	45 mg/kg	90 mg/kg
146.33±0.49	146.83±0.67	144.00±1.13	** 136.83±0.83	**135.00±0.33

Table 2. Absolute volume (mm^3) of cortex (C), medulla (M) and total of kidney (TK) in diabetic groups compared with control and sham control groups. Mean±SEM. **P<0.01

	Control	Sham Control	15 mg/kg	45 mg/kg	90 mg/kg
Cortex	475.17±7.50	480.00±9.57	**555.67±4.51	**657.33±9.11	**893.33±12.19
Medulla	391.66±5.02	391.33±4.97	388.50±7.24	395.33±9.04	**568.67±8.72
Kidney (total)	866±10.05	871.33±7.90	**944.17±4.34	**1045.33±8.12	**1462.00±17.19

Table 3. Total volume (mm^3) and total number of glomeruli in diabetic groups compared with control and sham control groups. Mean±SEM. * P<0.05, **P<0.01

	Control	Sham Control	15mg/kg	45mg/kg	90 mg/kg
Absolute volume	11.87±0.25	12.01±0.20	*13.03±0.16	**15.13±0.17	**19.00±0.35
Total number	31403±257	30673±226	31102±221	31080±215	30717±170

appears as increment in renal volume particularly at cortical zone is due to mesangial and capillary expansion as well as vascular and interstitial lesions. In short period contrary to long period of diabetes, destructive changes in parenchyma do not have effects such as occluding and obliterating glomeruli. Under normal circumstances both glomerular and tubular compartments of the kidney are composed of quiescent cells. Glomerular cell replication increases during the early phase of Streptozotocin induced diabetes mellitus as well as in several experimental models of glomerulosclerosis such as compensatory renal growth (29). In accordance with the present experiment, other studies have proved that in short period Stereptozotocin induced diabetes in rats the cortical tissue volume and glomerular volume increment did not compensate the loss of injured nephron. It seems that there is an imbalance between cell proliferation and cell apoptosis in the early course of diabetes. It should be considered that short period administration of more STZ might have more effects and cause more imbalances in cells turnover. The results of the present study are in accordance with other qualititative and quantitative surveys. Obviously, stereology is applicable as a good monitor of changes in kidney parenchyma and can be used as a powerful and efficient method in diagnosis and prognosis of kidney disease and in the course of diabetes. Moreover, it should be considered that in the early stage of diabetes mellitus induced by STZ, the inducer has different effects and more doses may cause more induction and have more effects on the kidney.

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Correspondence:.Zahra. Heidari, Department of Histology, Faculty of Medicine, Zahedan University of Medical Sciences, Iran. E-Mail: histology_iri@hotmail.com

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