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Structural Changes in Tunica Mucosa Cells of Bladder in Rats with Experimental Diabetes Mellitus

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Abstract: Diabetes mellitus causes some changes in urine quality and quantity. Therefore, we planned this study to explain how these changes affect the histological structures in the urinary bladder. We classified this experimental subjects under 4 Groups as follows; Group I: Control group, Group II: 60 mg kg^{-1} single dose of streptozotocin was administered intraperitoneally (i.p.), Group III: 40 mg kg^{-1} dose of streptozotocin i.p. was administered 5 days consecutively, Group IV: 150 mg kg^{-1} single dose of alloxan was administered i.p. We obtained the biopsy materials of tunica mucosa of the urinary bladder after 6 months, then we exposed these materials for observations with light and electron microscopy. In Group II, we observed a decrease in the number of urothelium layers, loss of dome shaped cells which were replaced with the intermediate layer cells. In Group II and especially in Group III, we observed an increase in the thickness of the epithelial and capillary basement membranes. In Group IV, we observed a repair process in urothelium. The increase in the thicknesses of the basement membranes were less than the other groups. After the 6 months of experimental period, the degeneration of tunica mucosa in Group III, which had the highest blood glucose levels, seems to be related to the complications of lasting diabetes. The mild degeneration of tunica mucosa in Group II and Group IV seems to be related to the insuline production which was caused by ß cell regeneration or island cell adenoma and also these factors reduced the complications of diabetes seriously.

Key words: Diabetes mellitus, bladder, tunica mucosa, streptozotocin, alloxan

INTRODUCTION

Neuropathy, polydipsia, polyuria, bladder disfunction, bladder enlargement, urinary retention, smooth muscle hypertrophy are the major complications of diabetes mellitus. It causes infections in the whole urinary system, especially in the bladder. The bladder expands when polyuria appears during the sickness process[1,2]. The increased cholinergic neuron activity causes hypertrophy of detrussor muscle[2-4].

Alloxan is a diabetogenic agent and its effects are caused by the necrosis process of ß cells in Langerhans Islands[5,6]. The second diabetogenic agent was found by Dounod *et al.*[7] which administered streptozotocin to cats and dogs intravenously.

The diabetogenic dose of streptozotocin is 60 mg kg^{-1}. Gajdosik *et al.*[8] reported that single dose of 50-60 mg kg^{-1} streptozotocin causes irreversible hyperglycemia and the single dose of 70 mg kg^{-1} streptozotocin is lethal.

Like and Rossine[9], Cossel *et al.*[10] showed that the injection of repeating doses of streptozotocin is an experimental model for Type I diabetes mellitus of human. Immun responses have roles in diabetes development.

Previous research showed that most of the studies were made about examining the tunica mucosa layer of the bladder, but the changes in tunica mucosa has not been studied yet. We planned to obtain several diabetes models by using different experimental methods.

In this study, trigon part of the bladder was extracted from the study because of different histological structure and embryological development.

MATERIALS AND METHODS

In this study Wistar Albino male rats under the same biological and physiological conditions were selected. We distributed rats under 4 Groups as follows: Group I: Control group, 5 rats; Group II: single dose of 60 mg kg^{-1} streptozotocin (SIGMA) was administered i.p.

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to 8 rats; Group III: Consecutive doses of 40 mg kg^{-1} streptozotocin (in pH= 4.2 and 0.1 M sitrat buffer solution) was administered to 8 rats for 5 days; Group IV: Single dose of 150 mg kg^{-1} alloxan (dissolved in 0.09% NaCl solution) was administered to 8 rats.

We measured the body weights and blood glucose levels by sampling tail venous blood of all rats before starting the study. We monthly measured the body weights and blood glucose levels for 6 months during the study. The biopsy materials were obtained from the tunica mucosa layer of the bladder in the end of sixth month.

For light microscopy, we fixated biopsy materials using Bouin fixative, then embedded into paraffin blocs. After these steps, we obtained 5 μm of sections and stained them with H+E, H+Von Gieson, PAS+HL, Masson to show the histological structure of the tunica mucosa. For electron microscopy, we used araldite fixative and stained our sections with urinal acetate- Reynold's leaded sitrat stain.

RESULTS

Group I findings: The biopsy materials which were obtained from the control group were examined under light and electron microscopy. All of the histological components of the tunica mucosa were found normal.

Group II findings: We examined the biopsy materials which were obtained from Group II under light microscopy and saw a decrease in number of the epithelial cells which form the urothelium. The crusty over the dome shaped cells was destructed and lost, according to this, most of the cells were shed to lumen because of the toxicity of urine and broken intercellular junctions. The cells which were connected to the basement membrane were prismatic shaped and the loss of the cytoplasmic material was obvious. We noticed that the number of the intermediate layer cells was decreased and both the cell and nuclear shapes were changed. Especially the cytoplasmic losses in perinuclear region of the cells were important (Fig. 1).

As a result of present observations under electron microscopy, the superficial layer of the cells were shed according to polyuria and the changes of urine pH which were caused by diabetes. These cells were replaced with the intermediate layer cells and even with the basal layer cells. The cells which were seen in the superficial layer, showed disc and disc like formations to protect their selves from the toxicity of urine.

The basal layer cells had less microfilaments than the intermediate layer cells and were not able to form Golgi originated vesicles enough. Besides, the intermediate layer cells, which formed superficial layer, had enough microfilaments and Golgi originated vesicles in their cytoplasms. The desmosomes between cells were ruined, the interdigitating distances were broadened and cell promontories were far and thick. The basement membrane was thick in some places and contained an electron dense material (Fig. 2).

Group III findings: The urothelium was consist of almost one layer. The dome shaped cells were lost and the superficial layer cells formed curves, but these cells did not have a crusty like feature. There was an evident cell infiltration in the connective tissue.

The first evident finding under electron microscopy was the loss of the dome shaped cells. The intermediate layer cells, which were replaced with the superficial layer cells, were in a process of forming disc and disc like systems which were lost, but this process was not rapid enough, so these cells were getting effected from urinary toxicity and loosing organelles, reducing cytoplasmic material production, having cytoplasmic spaces and all of these events resulted in shedding of the cells. The desmosomes and interdigitating distances between epithelial cells were broadened and thickened (Fig. 3). The basement membrane was thick in some places and contained an electron dense material. The connective tissue cells were activated in the lamina propria and the fibers were increased in number.

Group IV findings: When we observed the biopsy materials under the light microscopy, we met a tunica mucosa which was similar to the control group. Despite the loss of cell layers, lamina epithelialis generally contained 3 or 4 epithelial layers. Dome shaped cells were generally seen in the superficial layer, but in some places the absence of dome shaped cells was seen and most of the superficial layer cells did not have a crusty. The intermediate layer cells still had their polygonal shape, but their stratified architecture was lessened when compared with the control group. The basal layer cells had a prismatic shape and appeared to be activated. The basement membrane was thin and folded, the capillaries beneath were seen like almost they participated into the epithelium. There were many capillaries and evident cell infiltration in lamina propria.

When we observed the biopsy materials under electron microscopy, we saw a healing process in the urothelium (Fig. 4).

Some of the dome shaped cells were ruined because of diabetes and they were replaced with the intermediate layer cells. Some of these new cells were not differentiated enough to protect their selves from urine and these cells

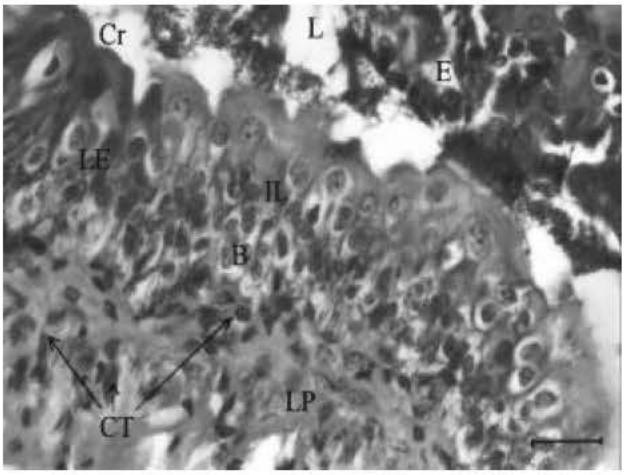

Fig. 1: Many changes are seen in lamina epithelialis and lamina propria. LE: Lamina Epithelialis, LP: Lamina Propria, L: Lumen, B: Basal Cells, IL: Intermediate Layer Cells, Cr: Crusty, CT: Connective Tissue Cells, E: Epithelial Cell Losses. Masson, bar 10 μm

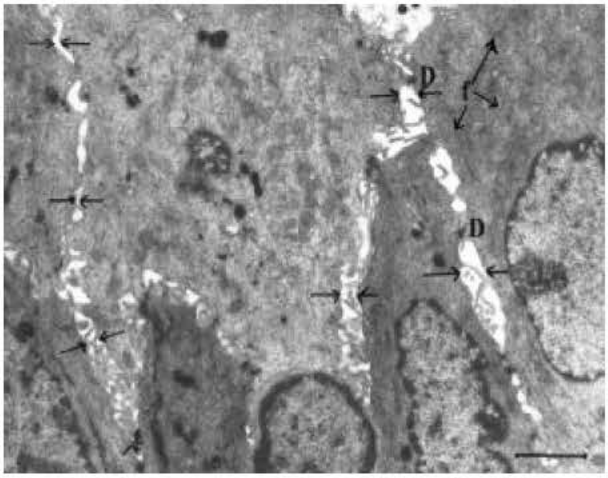

Fig. 3: The differentiations of the junctional complexes between epithelial cells are seen. D: Desmosome, →←: Interdigitation, f: Filament, bar 2 μm

were degenerated, but some of them were producing Golgi originated vesicles for adaptation. There were filaments near these vesicles in the cytoplasm. The mitochondria of the degenerated cells were expanded and the crista was thinned according to this degeneration. Junctional complexes and interdigitations were found normal in most of the epithelial cells (Fig. 4).

The basement membrane was thin and folded. There were many capillaries and cell infiltration in the lamina propria.

DISCUSSION

Diabetes mellitus causes disfunction, volume augmentation and smooth muscle hypertrophy in the bladder by its' complications. Eighty percent of diabetic patients have residual urine in the bladder because of insensitivity to the distantion[1,2,4].

The blood glucose levels may start to decrease a few months after the experimental diabetes is obtained according to the ß cell regeneration or island cell adenoma. The frequency of the island cell adenoma development is 5-99% after 1 year of streptozotocin induced diabetes model and this frequency is 90% in alloxan induced diabetes model between one month and one year[11].

In Group II to which we administered 60 mg kg^{-1} single dose of streptozotocin i.p., the blood glucose levels increased until the third month of injection, stayed constant until fourth month of injection, then started to decrease and finally decreased to normal levels in the end of the sixth month of injection.

In our opinion, this event may be caused by the ß cell degeneration and later the regeneration process of these cells or an island cell adenoma and this opinion shows parallelism to the studies of Bell and Hye[11].

The morphological studies indicates that the increase in thickness of the basement membrane is caused by diabetes and aging[12].

After observing Group II biopsies, we think that the increase in thickness of the basement membranes of the epithelium may be associated with diabetes.

In the biopsy materials of this group, the increase in thickness of the capillary basement membranes of the lamina propria in the bladder is similar to the studies of Kilo[4]. We think that the decrease of the number of cell layers which form urothelium may be associated with the increase of urine volume and the changes in urine concentration which applies pressure to the cells and the characteristics of the exposure material which these cells are exposed. The epithelium layer is nourished from the capillaries beneath the thickened basement membrane, thus we think that some nutritional materials may have some difficulties when passing through the basement membrane and this causes morphological changes in the epithelial layer.

On the other hand, we think that apical sides of the dome shaped cells are ruined and dejenerated because of the urine toxicity and the cellular activity is decreased according to the increase in the thickness of the basement membranes, so Golgi apparatus closed vesicle production ability is decreased. In our opinion, these closed vesicles are insufficient to form apical side of the cell membrane, so the loss of antiadherent factor of the cell causes a greater damage from urine in the lumen, the junctional complexes between cells are loosened and the cell is degenerated. We saw that the basement membrane stimulated polarities and differentiation of the adjacent cells to produce closed vesicles in order to have a function like dome shaped cells. After (fourth month), as seen in our findings, we suggest that obtaining constant and normal blood glucose levels may result in normal thickness of the basement membranes and this may cause an acceleration in healing of the urothelium.

However, when we obtained the biopsy materials, the blood glucose levels had just decreased to normal levels, so we believe that the thickness of the basement membranes could not reach to normal values and the intermediate layer cells which were trying to form like dome shaped cells, could not differentiate completely, by the way the intermediate cells were decreased in number.

We think that the connective tissue augmentation in the lamina propria is caused by the fibers which revealed to enhance poorly nourished connective tissue because of the thick basement membranes and migrated infiltration cells against the changes which happens in the urothelium after the loss of the dome shaped cells and anti adherent factors.

We realized that the rats in Group III to which we administered 40 mg kg^{-1} dose of streptozotocin for 5 days consecutively had an increase in body weights until the third month when the blood glucose levels reached 400-450 mg dL^{-1}, but from the end of the third month to the end of the sixth month the blood glucose levels stayed constant. We think that in Group III, diabetes was originated from the ß cell destruction or loss, as understood from the blood glucose levels and this caused the complications of diabetes. We believe that, in biopsy materials, the presence of almost one layer of the lamina epithelialis in the bladder was caused by the increase in thickness of the basement membrane which is one of the demain complications of diabetes and the destruction of the apical sides of the dome shaped cells which were exposed to urine in the lumen. The increase in thickness

of whether capillary or urothelium basement membranes effected the nourish and oxygenation of the epithelial cells, so the cell metabolisms were predicted to slow down. During this period, the urine, effected the crusty which had a role as an anti adherent factor, meanwhile the dome shaped cells which had slowed metabolisms were not be able to repair the crusty rapidly, so after the destruction of the crusty the dome shaped cells were effected by urine and they underwent a greater degeneration. We suggest that the loss of organelles, the loss of cytoplasmic concentration and increased distance between the junctional complexes designate these situations.

We suggest that the infiltration cells which were seen in the lamina propria, appeared to protect the tissue after the loss of the anti adherent factors.

In our opinion, the increased fibers in the connective tissue were produced by the connective tissue cells against the loss of the connective tissue which was caused by the increase in thickness of the basement membrane.

In Group IV, the blood glucose levels increased during the four months, but then the levels began to decrease and at the end of the sixth month, it were almost normal.

In Group IV, although the tunica mucosa didn't have a completely normal histological structure, we saw dome shaped cells containing crusty, almost normal intermediate and basal layer cells because of ß cell regeneration and adenoma formation.

In Group IV, the causes of the findings which we saw in whether lamina epithelialis or lamina propria elements in tunica mucosa may be explained in the same way like the findings of Group II.

As a result of the changes in quality and quantity of urine which is caused by diabetes, the crusty in the tunica mucosa of the bladder is completely destroyed in type I diabetes and ruined in type II diabetes, so it's important for physicians that this creates a predisposition to several infections and various pathological problems. Also, in addition to lots of complications, the high blood glucose levels causes an increase in thickness of the basement membranes and demolish the nourish of the tissues.

In addition, in the studies which are being planned at the moment for more than four months using streptozotocin and alloxan, it's necessary to repeat the doses of these agents or evaluations should be made with respect to this point.

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