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ULTRA-STRUCTURAL ABNORMALITIES OF THE URINARY BLADDER IN STREPTOZOTOCIN-INDUCED DIABETIC FEMALE RATS.

Hypothesis / aims of study

Diabetic cystopathy is the commonest form of peripheral autonomic neuropathy and one of the most frequent complications of diabetes mellitus [1]. Autonomic neuronal degeneration and voiding dysfunction in diabetes mellitus are known but the morphological changes in the detrusor muscle have not been adequately investigated or correlated to the duration of diabetes [2]. Ultra-structural morphological lesions were however demonstrated in urinary bladder biopsies of non-diabetic patients with neurological disorders and were correlated with the urodynamic diagnosis of abnormal lower urinary tract function [3]. Because of the limited epidemiological data on diabetes mellitus and voiding dysfunction, there is a unique opportunity to address further the association and the potential mechanisms that interrelate diabetes and this disorder in animal models [1,2]. Therefore, the objective of this study was to evaluate the ultra-structural changes in the urinary bladder of female diabetic rats in relation to disease duration and severity.

Study design, materials and methods

Adult female Wistar rats (weight= 200-225 g, age= 3 months old, n=30) were used in this study. Diabetes mellitus was induced in the study group (n=18) by a single intra-peritoneal injection of 60 mg/kg body weight of streptozotocin (Sigma Chemical Co., St. Louis, Missouri, USA) in citrate buffer (0.1M, pH 4.5). The age- and weight- matched control animals (n=12) were injected intra-peritoneally with a proportionate volume of buffer alone (n=6) or were left untreated (n=6). Diabetes was confirmed 24 hours later by the presence of a plasma glucose concentration of > 6.7 mmol/l using a standard glucometer. Both control and experimental animals were euthanised by excess ether anaesthesia at 8 (n=6), 12 (n=6), and 16 (n=6) weeks post-treatment and urinary bladder specimens were obtained, fixed in McDowell and Trump fixative and processed for electron microscopy. The epithelium, stroma, detrusor muscle and autonomic nerve plexus were examined. Severity of experimental diabetes mellitus was monitored by analysis of blood glucose levels in each study group before euthanasia. The Animal Research Ethics Committee approved the study protocol.

Results

Distinct ultra-structural changes were demonstrated throughout all layers of the urinary bladder in diabetic female rats compared to controls. The superficial aspects of epithelial cells presented numerous multi-lamellate vesicles in the apical and lateral marginal cytoplasm. Extensive portions of the intercellular junctions appeared folded and distorted. Dispersed ribosomes, a few strands of rER, plenty of mitochondria and heterochromatic nuclei were other features. The basal epithelial cells had abundant ribosomes, rER, and procollagen fibrils with presence of intraepithelial capillaries and basolateral collagen accumulation. The muscle tissue comprised electron lucent and electron dense myocytes. The cell junctions were extensively widened and the intercellular space contained collagen, ground substance and often myelin bodies. Both the stromal fibroblasts and smooth muscle cells appeared to be producing large quantities of collagen. Some capillaries had plenty of pinocytic vesicles while others did not have such vesicles. The nerve terminals were surrounded by thick ground substance and contained dark mitochondria and dense whorls. The most prominent ultra-structural changes in the diabetic group, therefore, were the presence of intraepithelial capillaries, basolateral collagen accumulation, increased number of mitochondria in the transitional epithelium, excessive production of collagen by both the stromal fibroblasts and detrusor myocyte, widening of the cell junctions between these myocytes (detrusor dysjunction) and extensive degeneration of the serosal nerve fibres. All of these structural abnormalities were progressive with longer duration and severity of diabetes.

Interpretation of results

The ultra-structural pattern of lesions identified in the detrusor muscle of our diabetic group concurred with muscle dysjunction described in non-diabetic patients with neurogenic bladder

dysfunction who had detrusor overactivity [3]. This indicates that detrusor overactivity may be the main clinical feature of diabetic cystopathy. Excessive accumulation of collagen in these myocytes might also cause impaired detrusor contractility. The severity of detrusor lesions was dependant on the duration and the severity of diabetic insult [2]. The abundance of epithelial mitochondria in the urinary bladder of diabetic animals is consistent with increased oxidative stress suggesting that this mechanism may be primarily involved in the pathogenesis of diabetic cystopathy [1]. All our experiments were performed in female rats. However, there is no evidence of sex-related differences in the structural changes that may occur in the urinary bladder of streptozotocin-induced diabetic rats.

Concluding message

Although a direct extrapolation of laboratory data to clinical situation has limitations, morphology-dependent diagnostic methods such as bladder biopsy may be useful in detecting asymptomatic lower urinary tract dysfunction in diabetic patients with a more positive impact on management. This is particularly important in populations with a high prevalence of diabetes mellitus. Histological examination of the bladder in the diabetic state may also provide a better understanding of the role of diabetes mellitus in voiding dysfunction.

Key Words

Detrusor muscle, experimental diabetes mellitus, rats, ultra-structure, urinary bladder, voiding dysfunction.

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