Proceedings of International Conference on Technology and Social Science 2017

Early Effects of Streptozotocin Diabetes on Capillaries in Spinal Cord

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Keywords: Diabetic microangiopathy, neuronal complications, spinal capillary

Abstract. Diabetic microangiopathy is a well-known cause of neuronal complications mainly in peripheral nerves and brain. Additionally, it also strongly associates with the development of spinal infarction followed by the physical disability to seriously affected the quality of life in diabetic patients. However, only few study is known about the alterations of the spinal capillary in diabetes. Therefore, this study aimed to illustrate morphological changes of spinal capillaries in the early stage of streptozotocin (STZ)- induced diabetic rat compared with control. In diabetes, the endothelial cell and pericyte became hypertrophy. Under the ultrastructural level, the endothelial cell contained a vast number of ribosomes and vesicles, enlarged mitochondria, and disrupted tight junction, while the vascular pericyte had numerous free ribosomes and a pair of centrioles. Furthermore, basement membrane thickening was detected. This basic knowledge will be beneficial for raised awareness and receiving in early diagnosis of the spinal vascular disorder in diabetic patients.

1. Introduction

During diabetes mellitus (DM), a very sensitive target to high glucose is the blood vessel. Hyperglycemia initiates the pathogenesis of macroangiopathy and microangiopathy [1], causing insufficient blood-induced dysfunctions of the several organ systems. In many studies, the alterations of capillary components; endothelial cell and pericyte in the peripheral tissues and the nervous tissues are investigated. In the early stage of diabetes, the retinal capillary cell death caused retinopathy [2], as well as the glomerular endothelial cell proliferation with thickened basement membrane occurred in relation to progressing histological lesion in nephropathy [3]. Regarding the severe diabetic neuropathy, the nerve capillary pathology has also been studied, for instance, a decrease in endoneurial capillary density, an increase in thickening of total diffusion barrier and a reduction of oxygen diffusing capacity [4]. In the cerebral microvessels, the vascular lumen stenosis, thickened vascular basement membranes, endothelial cell swelling, and the shedding has been revealed in the STZ- induced diabetic rats [5]. Furthermore, one cause of disabling event in the patient with diabetes have also been reported with the abnormal vascular wall and the embolic occlusion in a vessel leading to an inadequate blood circulation and subsequent degenerative changes in the spinal cord. As the spinal cord is the center route to carry nerve impulses, the clinical presentations commonly relate to the sensory deficit, progressive weakness symptoms, and autonomic control disturbance, corresponding to the levels of the cord lesion [6], [7]. However, few studies examine spinal capillaries, especially the architecture of diabetic vasculopathy. Then, this study focused on the histological and ultramicroscopic examinations of spinal capillaries in the short duration STZ- induced DM.

2. Materials and Methods

2.1 Animal preparation

Fifteen male Sprague-Dawley rats (5-8 week old, 200-270 g body weight) were provided by the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. Rats were taken care in accordance with the Mahidol University Care and Use of Laboratory Animal.

2.2 Experiment protocols

Rats were randomly divided into two groups: control (n=6) and diabetic animals (n=9). Type 1 diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg body weight) dissolved in citrate buffer (pH 7.4). The control rats received only the citrate buffer. After STZ administration for 48 and 72 hours, the blood samples were collected from the tail vein and the blood glucose level was measured by a glucose meter (OneTouch® Ultra®, Colorado, USA). Rats with ≥300 mg/dL blood glucose level with symptoms of polyuria, polyphagia, and polydipsia were considered as diabetes and included in this study. At the end of the experiment (4 weeks after the induction), the animals were measured the blood glucose levels before sacrifice and anesthetized by halothane inhalation. Whole animal perfusion through the heart was processed for histological and electron microscopic examinations.

2.3 Histological study

The 0.9% NaCl perfusion was performed, followed by injection of Bouin's solution to preserve tissues. Next, the spinal cords, which were separated into cervical enlargements (C), thoracic segment (T), lumbosacral enlargements (L), were harvested and immersed in the same fixative overnight. After rinsing with 70% ethanol several times, these specimens were dehydrated in a graded series of ethanol, cleared in xylene, infiltrated, and embedded in paraffin. Serial cross sections were cut with a microtome (Leica RM 2035, Wetzlar, Germany) at 6 μm thickness, mounted on glass slides and stained with the routine hematoxylin and eosin (H&E) technique. Photography and morphometric studies were done under a light microscope (LM) (Axiostar plus, Jena, Germany) connected to a digital camera (AxioCam MRc, Jena, Germany).

2.4 Transmission electron microscopy

The 0.1 M phosphate buffered saline (PBS) solution was perfused through the heart and subsequently injected with 2.5% glutaraldehyde in 0.1 M PBS to fix the tissues. The spinal cords including C, T, and L were removed and cut into small cubes. The tissue was postfixed in 0.1% osmium tetroxide, dehydrated in ethanol series, infiltrated in propylene oxide, and embedded in the plastic. The ultrathin sections were cut with an ultramicrotome (Leica EM UC6, Vienna, Austria), mounted on copper grids, counterstained with 1% acetate and lead citrate, and observed under the JEOL JEM 100S transmission electron microscope equipped with a digital camera (Tokyo, Japan).

3. Results

3.1 Histology of spinal capillary

Similar alterations during early diabetic stage were showed in the spinal capillaries in each area of gray and white matters from all spinal levels (C, T, and L). Under the LM, the spinal cords consisted of neurons, nerve fibers, neuroglia, and capillaries. Concerning to the spinal capillaries, they occupied among neurons and neuroglia in the gray matter, while they paralleled to nerve fibers and neuroglia in the white matter. Each spinal capillary was formed by two cell types; endothelial cell and pericyte. The endothelial cell had a flat basophilic nucleus enclosed spherical-shaped capillary lumen. The prominent nucleus of pericytes capped on the basal side of endothelial cell layer (Figs. 1A, 1C). In the DM, both endothelial cells and pericytes contained larger nuclei than those in the control rats. (Figs. 1B, 1D).

3.2 Ultrastructure of spinal capillary

In the ultramicroscopic analysis of normal capillaries, the endothelial cell had a single flattened endothelial nucleus and a thin layer of cytoplasm surrounded a large round capillary lumen. Endothelial nucleus contained electron-lucent euchromatin and electron-dense heterochromatin (Fig. 2A). A large number of mitochondria inside the spinal endothelial cell were visible as a rod or spherical shape bounded with the double membrane layers. Rough endoplasmic reticulum (rER) displayed the slimline cisternae attached by numerous dark spots of ribosomes. Between endothelial cell and pericyte, the basal surface of the endothelial cell was underlined by the thin fibrillar layer of basement membrane (Fig. 2B). In the diabetic capillaries, there were numerous free ribosomes scattered within a large volume of cytoplasm together with a group of short rER with dark stained elements. Basement membrane thickening in capillaries was demonstrated in figure 3A. A marked enlargement of mitochondria with cristae disruption was observed (Fig. 3B). Moreover, there were a large vacuole and a lot of vesicles in the cytoplasm near the basement membrane and subplasmalemmal zone (Figs. 3A-C). Apart from the endothelial cell, pericyte was also defined as the vascular cells. It became enlarged in diabetes and contained a large number of electron-dense spots of free ribosomes, particularly in the perinuclear region. The long cisternae of rER and lightly stained mitochondrial matrix with a few cristae also presented. In addition, a pair of tube-shaped centrioles appeared near the nuclear membrane (Fig. 3D).

In the endothelial tight junction (TJ), the electron-dense line on the plasma membrane of adjacent cells was structurally intact in the normal condition (Fig. 4A). During diabetes, the alteration of TJ was the imperfect anchoring of the unit membrane, leading to the small gap formation between two adjacent endothelial cells. Additionally, a reduced electron density of the TJ site occurred in diabetes (Fig. 4B).

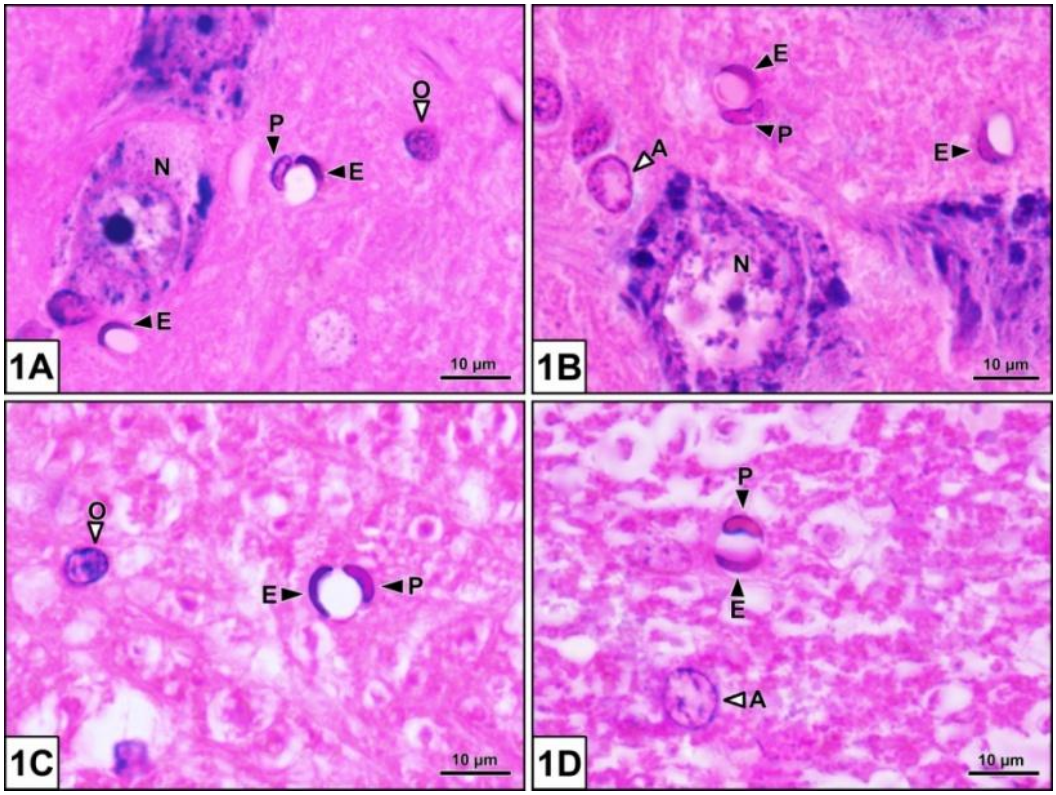

Fig. 1. Light micrographs of the spinal capillaries in gray (1A, 1B) and white matters (1C, 1D) of the spinal cords in control (1A, 1C) and diabetic (1B, 1D) rats. In the diabetic capillary, the enlargement of endothelial cells and pericytes were found. Endothelial cell (E); pericyte (P); neuron (N); oligodendrocyte (O); astrocyte (A). H&E staining.

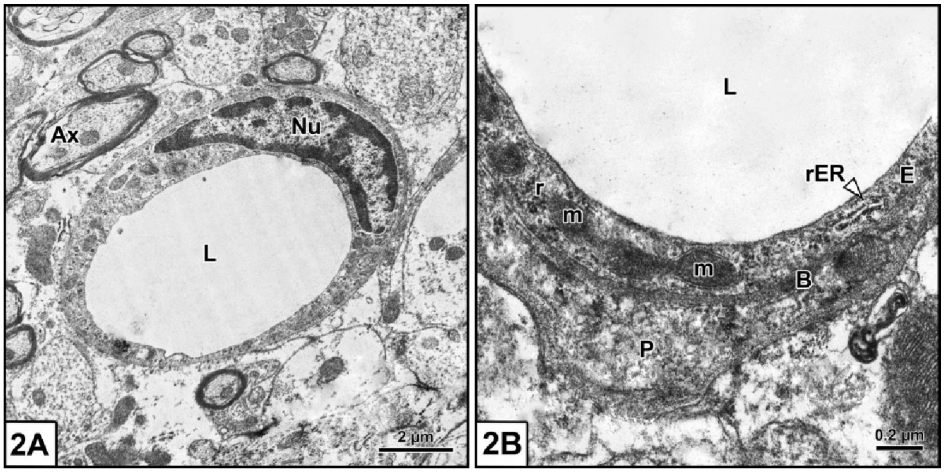

Fig. 2. In the normal appearances of the capillary, the flattened nucleus (Nu) of the endothelial cell enclosed the spherical lumen (L) (2A). With higher magnification, cytoplasmic organelles in the cytoplasm of the endothelial cell (E) contained mitochondria (m), ribosomes (r), and rough endoplasmic reticulum (rER) (2B). Axon (Ax); basement membrane (B); pericyte (P).

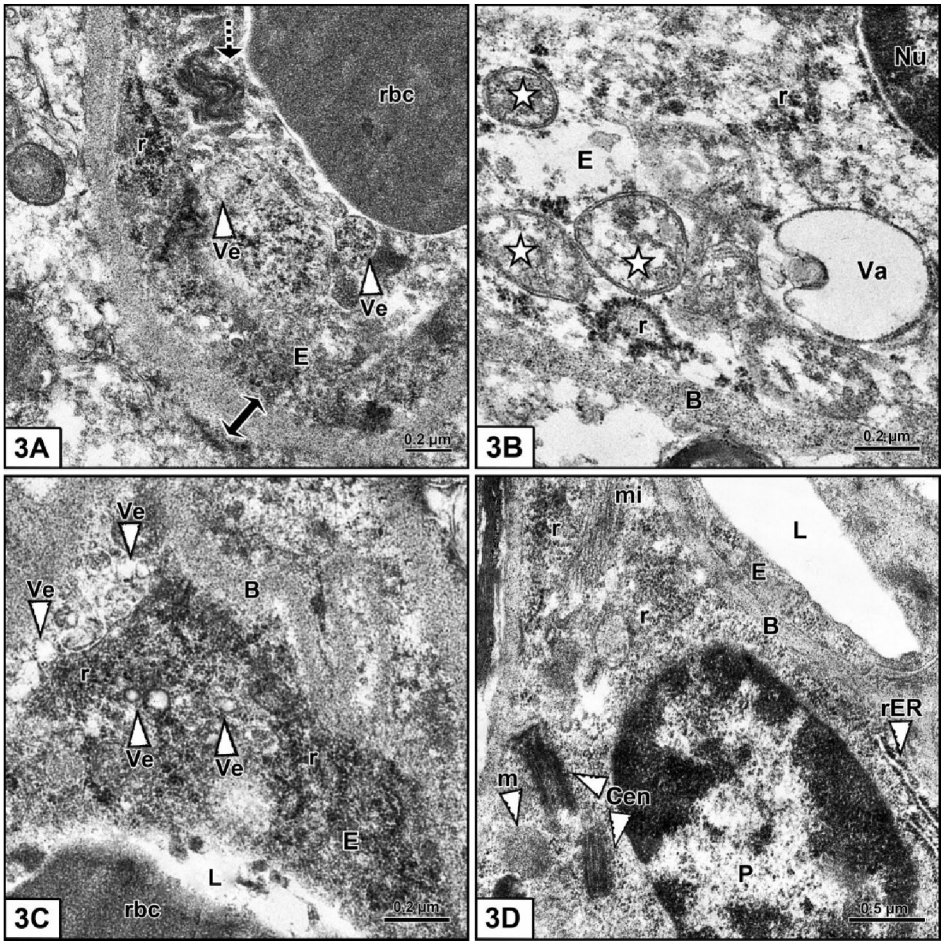

Fig. 3. Cellular injuries of the diabetic spinal capillaries were found in endothelial cells (3A-C) and pericyte (3D). In the diabetic endothelial cell (E), thickened basal lamina (a two-headed arrow), cluster of ribosomes (r), dense material group of short rough endoplasmic reticulum (a dashed arrow) in 3A; dilated mitochondria (white stars) and a large vacuole (Va) in 3B, a lot of endocytic vesicles (Ve) in 3C were showed. Additionally, the diabetic pericyte (P) became edematous with a cluster of ribosomes (r), mitochondria (m), rough endoplasmic reticulum (rER), microfilaments (mi), and a pair of centrioles (Cen) as shown in 3D. Red blood cell (rbc); capillary lumen (L); basement membrane (B); endothelial nucleus (Nu).

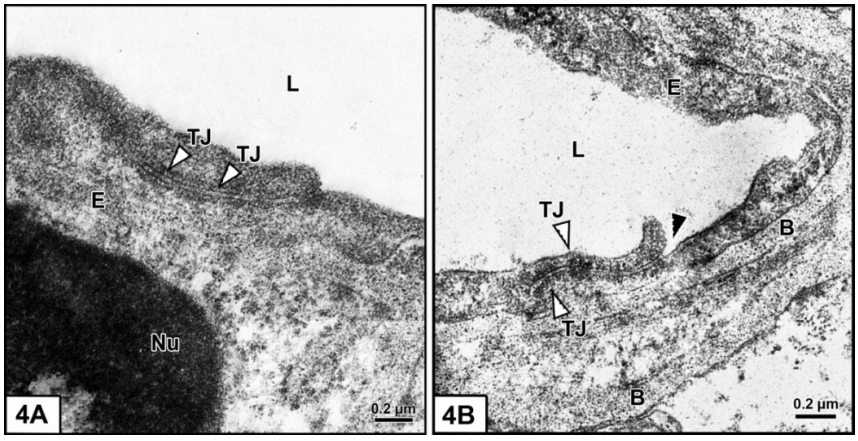

Fig. 4. Transmission electron micrographs of tight junction (TJ) interlocking between endothelial cells of the spinal capillary in the control (4A) and diabetic (4B) rats. In the normal tight junction, intact electron dense line was elucidated (4A), whereas detachment of TJ (a black arrowhead) appeared in the DM (4B). Endothelial cell (E); nucleus (Nu); basement membrane (B); capillary lumen (L).

4. Discussion

The development of diabetic microvascular complications in the spinal cord occurred in the early phase of diabetes. The endothelial cell lining of the capillary lumen and the pericyte wrapping around the endothelial cell of the capillaries increased in their sizes. The excess glucose in these cells during diabetes can be converted into the sorbitol and fructose via the polyol pathway contributing to an increase in intracellular oncotic pressure [8]. This change results in water entering the cell causing cellular edema. Moreover, the high glucose level can induce transcellular transportation. During diabetes, advance glycation end product (AGE) induces an increase in vascular endothelial growth factor (VEGF) expression through nuclear factor kappa B (NF-Kβ) pathway. Then, VEGF can increase the endothelial permeability via an endothelial nitric oxide synthase (eNOS)- the dependent process of transcytotic transport in plasma membrane caveolae through a mechanism that involves the phosphoinositide 3 kinase (PI3K)- protein kinase B (Akt)- eNOS signaling [9]. Therefore, the formation of numerous interconnected membrane invaginations, termed the vesicles and large vacuoles, was also found in the cytoplasm of diabetic endothelial cells. Furthermore, an increased VEGF robustly activates the PI3K-Akt pathway. The phosphorylation of the Akt targets contributes to cell survival, growth, and proliferation. Thus, abundant free ribosomes and duplicated short rER, that were essential for protein synthesis during the interphase of the cell cycle, were observed in the cytoplasm of the diabetic endothelial cell. In addition, a pair of centrioles for cell division was seen in the pericyte near its nucleus. It was concluded that AGE in hyperglycemic environment stimulates aberrant angiogenesis through the angiogenic peptide VEGF [10]. VEGF directly contributes to capillary basement membrane thickening via increased type IV collagen deposition in diabetes [11]. Moreover, the glycation products themselves can induce gene expression and synthesis of tumor necrotic factor alpha (TNF-α) in the endothelial cells [12]. The TNF-α represses the claudin 5, an integral membrane protein which is a critical component of endothelial TJ, via protein kinase C/NF-Kβ signaling pathway in diabetic condition [13]. Thereby, the detached TJ occurred in the diabetes, implying a vascular permeability defect. The reported alterations of spinal capillaries in early stage of diabetes in this study should persist to fully elucidate the short-term effects of diabetes type 1 on spinal microvascular dysfunction in order to better early therapeutic detection to reduce chronic diabetic complications.

5. Conclusion

Early developed diabetes causes the spinal capillary changes including the endothelial cell lining of the capillary lumen and the pericyte wrapping around the endothelial cell. The capillary abnormalities may be suggested as a possible pathogenic cause of diabetic neuropathy in latter. An

early detection of spinal capillary lesion gives the chance to reduce the severity of microangiopathy related to neuropathy in the diabetic patients.

Acknowledgements

This study was supported by the Siriraj Graduate Scholarships, Siriraj Research Fund, and Chalermphrakiat Grant, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

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