Ultrastructural evidence of peripheral neuropathy in spontaneous hyperglycemic Otsuka Long-Evans Tokushima Fatty rats: histopathological similarity to human diabetic neuropathy

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Abstract The ultrastructure in the peripheral nerves of spontaneous hyperglycemic rats and of two cases of human diabetic neuropathy was compared using electron microscopy. Experimental animals were Otsuka Long-Evans Tokushima Fatty (OLETF) rats, bred to reveal spontaneous hyperglycemia and glucosuria after 20 weeks of age. The ultrastructural findings in the peripheral nerves of OLETF rats consisted of destructive changes of the myelin sheath, followed by axonal degeneration and basal laminal thickening of Schwann cells covering small-caliber axons. These alternations in the endoneurium were associated with the later onset of capillary vasculopathy, which revealed duplication of endothelial basal lamina and proliferation of microvilli at the luminal surface. It was characteristic that these histological alterations were apparently distinguished according to the duration of hyperglycemia between 10 and 30 weeks of age. The two human diabetic cases, who had suffered from non-insulin-dependent diabetes mellitus (NIDDM) for a considerable period, had undergone amputation of the leg following the onset of severe neuropathic ulcerative necrosis. Biopsied specimens from peripheral nerves revealed perineurial thickening, proliferation of endoneurial collagen tissue, loss of myelinated fibers, extension of Schwann cell processes with occasional "onion-bulb" formation, and endoneurial vascular basal laminal thickening. The results of these comparative histological studies suggested that the alterations occurring in peripheral nerve tissue of experimental diabetic OLETF rats may reflect the early pathological changes of human diabetic neuropathy.

Key words Diabetic neuropathy · Basal lamina · Myelin · Axon · Vasculopathy · OLETF rats · Hyperglycemia

Introduction

Peripheral neuropathy is one of the most common complications of human diabetes mellitus. However, little is known of the structural alterations and pathogenic mechanisms responsible for the various clinical presentations of the neuropathy. Identical pathological features of diabetic neuropathy are nerve fiber loss, axonal degeneration, and segmental demyelination with or without some degree of associated microvasculopathy. The most probable pathogenic mechanisms that would lead to this characteristic neuropathy could be of vascular, axonal, or Schwann cell origin.

In considering experimental diabetic neuropathy models, an ideal animal model should demonstrate the early metabolic changes linked to abnormalities of the polyol pathway as well as the late degenerative changes associated with microangiopathy and ischemia and should provide evidence of degeneration in axons, myelin, and blood vessels as observed in human diabetic neuropathy. Various experimental diabetic animals have been used for the study of peripheral neuropathy, diabetes being induced via galactose intoxication, by streptozotocin, or by using BB-Wistar or WBN/Kob rats. However, unlike those in humans, these pathological changes in experimental neuropathy are varied from axonopathy to myelinopathy, which may be thought to be secondary to axonopathy, and the association with microangiopathy. It has also been mentioned that the neuropathy in experimental animals described in previous reports is indistinguishable from the aging process.

Recently it has been proved that the pathophysiological features in Otsuka Long-Evans Tokushima Fatty (OLETF) rats are quite similar to those in human non-insulin-dependent diabetes mellitus (NIDDM). However, it has not...
yet been investigated whether diabetic neuropathy as seen in human NIDDM might occur in OLETF rats. Therefore, the current study was designed to assess if there might be any pathological change found in peripheral nerves using OLETF rats, at the level of ultrastructural morphology, and then if this experimental model could reflect the structural disorders seen in human diabetic neuropathy. To answer this question, we investigated nerve specimens taken from both hyperglycemic OLETF rats in which peripheral neuropathy had been induced and diabetic neuropathic patients so as to assess comparative ultrastructural changes.

Materials and methods

Experimental animals

We used 20 male OLETF rats for this study. At the beginning of the experiment, they weighed 219–295 g and were 5 weeks of age. They were fed a standard diet with hyperosmotic water containing 20% sucrose. Body weight, blood glucose, and urine sugar levels were recorded every week. At the age of 10, 20, or 30 weeks, the animals were anesthetized by intraperitoneal injection of pentobarbital and perfused via the left ventricle with a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.2M cacodylate buffer, pH 7.4. Following the perfusion, tissue blocks were taken from sciatic, tibial, and common peroneal nerves at both sides, and fixed in fresh fixative overnight. These samples were washed in cacodylate buffer and postfixed in 2% solution of osmium tetroxide for 3h. After dehydration, tissue blocks were embedded in epoxy resin (Epok 812); 1-μm-thick sections were stained with toluidine blue. After light microscopic inspection, ultrathin sections were made from the selected areas and stained with 1% uranyl acetate and 0.1% lead citrate. Each specimen was observed with a JEM 1200 EX transmission electron microscope (JEOL, Akishima City, Tokyo, Japan).

Human biopsy specimens

Nerve biopsy specimens were taken from two human patients with diabetic neuropathy. Both patients were male, one being 53 and the other 78 years of age. Both patients suffered from severe neuropathic and angiopathic necrosis of the legs caused by chronic uncontrollable NIDDM. When below- and above-knee amputation, respectively, was performed, the femoral nerve and its peripheral branches were resected from the proximal side of the nerve trunk. Tissue blocks were promptly fixed in 2% glutaraldehyde, postfixed in 2% osmium tetroxide solution, and processed for an electron microscopic assessment as just described for rat tissues.

Results

Diabetic OLETF rats

Body weights and blood glucose levels of OLETF rats were significantly increased, and it was found that all animals developed hyperglycemia and glucosuria at 20 weeks of age (Table 1). At 10 weeks of age, under light microscopic inspection, the nerve fibers in the peroneal nerve trunk of rats appeared to be normal (Fig. 1a). There was no apparent loss of axons nor any evidence of degenerated nerve fibers. Electron microscopic study showed that each nerve fiber was covered by an intact Schwann cell, and the myelinated lamellae and microorganelles of axoplasm were clearly identified (Fig. 1b). At 20 weeks, the arrangement of endoneurial nerve fibers seemed slightly irregular. However, there was no evidence of an increase of degenerating nerve fibers in the endoneurium. Ultrastructurally, the presence of degenerative nerve fibers was detected. Degenerating axons contained myelin degradation products, and vacuoles were recognized (Fig. 2).

At 30 weeks, nerve fiber arrangement became more irregular, and endoneurial edema was apparently manifested as an increase in interstitial space around blood vessels (Fig. 3a). In the endoneurium, the degenerative change of nerve fibers was more clearly identified. In myelinated nerve fibers, accumulation of neurofilaments and clumping of microorganelles were recognized (Fig. 3b). In contrast, unmyelinated fibers seemed more affected. The basal lamina of Schwann cells covering small-caliber axons were multilayered. Intraaxonic vacuoles were also prominent in this period (Fig. 3b). Processes of Schwann cells covering unmyelinated nerve fibers became flattened and spicular (Fig. 3c). It was characteristic that strands of basal lamina, which were probably derived from degenerating Schwann cells, became detached and were freely present in the endoneurial interstitial space (Fig. 3c).

There was no evidence of degenerative change in endoneurial capillaries until 30 weeks. In 30-week-old rats, degenerative changes in blood vessels were found. Basal laminae covering the endothelium were proliferative and scaffolded, and a large quantity of microvilli were observed at the lumen of the endoneurial vessel. Endothelial cytoplasm became electron dense and flattened (Fig. 3d). The intercellular junction appeared to be tightly closed during the whole period.

Table 1. Final body weight and blood glucose level of 10-, 20-, and 30-week old OLETF rats (mean ± SD)

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of rats</th>
<th>Body weight (g)</th>
<th>Glucose level (mg/dl)</th>
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</thead>
<tbody>
<tr>
<td>10 weeks</td>
<td>4</td>
<td>413.5 ± 12.3</td>
<td>103.8 ± 6.1</td>
</tr>
<tr>
<td>20 weeks</td>
<td>6</td>
<td>637.5 ± 39.1</td>
<td>221.2 ± 27.9</td>
</tr>
<tr>
<td>30 weeks</td>
<td>7</td>
<td>638.3 ± 93.6</td>
<td>370.4 ± 83.8</td>
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OLETF, Otsuka Long-Evans Tokushima Fatty rats.