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Pathomechanism of entrapment neuropathy in diabetic and nondiabetic rats reared in wire cages

Toshiko Nishimura[1], Hitoshi Hirata[2], Masaya Tsujii[1], Ryu Iida[1], Yoko Hoki[1], Takahiro Iino[1], Satoru Ogawa[3] and Atsumasa Uchida[1]

[1]Department of Orthopaedic Surgery, Mie University Faculty of Medicine, Tsu, Mie, Japan,

[2]Department of Hand Surgery, Graduate School of Medicine, Nagoya University, Nagoya, Aichi, Japan and

[3]Electromicroscopy Research Center, Mie University Faculty of Medicine, Tsu, Mie, Japan

Summary. To examine the pathomechanism of entrapment neuropathy associated with diabetes with special emphasis on the roles of mast cells and Tenascin-C using a rat model of Streptozotocin-induced diabetes. The roles of mast cells and Tenascin-C in development of tarsal tunnel syndrome were analyzed electrophysiologically and histologically in 20 male Ws/Ws$^{-/-}$rats (mast cell deficient) and 20 of their male wild type counterparts (12-16 weeks old; 250-300g). Rats were assigned randomly to one of the following three groups; diabetic group and nondiabetic group reared in cages with a wire grid flooring; non-diabetic group in cages with sawdust covered plastic flooring. No significant role for mast cells in entrapment neuropathy was found in the rats with streptozotocin-induced diabetes. Distal latency was prolonged in diabetic rats compared with nondiabetic rats, and positively correlated with increases in blood glucose levels. Tenascin-C expression levels in the endoneurium at the tarsal tunnel in diabetic rats were found to be correlated with distal latency. The anti-alpha-smooth muscle actin (α-SMA) positive myofibroblast was scattered in nerve fascicles overexpressing Tenascin-C. It seems likely that Tenascin-C expressing myofibroblasts constrict axons by inducing collagen contraction of the endoneurium. Our data indicate that metabolic and phenotypic abnormalities of endoneurium and perineurum lie behind the vulnerability of diabetic patients to entrapment neuropathy.

Key words: Neuropathy, Pathology, Streptozotocin, Tenascin-C, Mast cell

Offprint requests to: Toshiko Nishimura M.D., Department of Orthopaedic Surgery, Mie prefectural Kusanomi Rehabilitation Center for Children 1-29-25, Shiroyama Tsu Mie, Japan 514-0818. e-mail: nishit41@pref.mie.jp

Introduction

Diabetic neuropathies are complex heterogeneous disorders that include both focal neuropathies and diffuse polyneuropathy (Consensus statement, 1988). Entrapment neuropathy is an example of a focal neuropathy, while distal symmetric polyneuropathy is the most common type of diffuse polyneuropathy. In addition to differences in distribution of these two types of neuropathy, there is a significant difference in pathology between the two disorders. The pathological features of entrapment neuropathy are focal demyelination along with a considerable reduction in caliber of the nerve at the site of entrapment, as well as bulbous swellings, most prominent proximal to the compression but also found distally (Lundborg, 1988). In contrast, features of distal symmetric polyneuropathy are apparent fiber loss or pronounced axonal degeneration (Dyck and Giannini, 1996). Common entrapment sites in diabetic patients are median, ulnar, radial, femoral, lateral cutaneous nerves of the thigh, peroneal, and medial and lateral plantar nerves. Although diagnosis of entrapment neuropathy in a nondiabetic is easily confirmed with electro-physiological tests, knowing where entrapment ends and polyneuropathy begins can be problematic in diabetics. Therefore, accurate prevalence figures are difficult to determine, and focal compression mononeuropathies at sites of entrapment are suspected to be a more common complication of diabetes than polyneuropathy (Zochodne, 1999). Despite the extensive clinical and experimental research over the last decade, the reasons why diabetic nerves are susceptible to entrapment are not well understood (Dyck et al., 1989; Zochodne, 1999).

Various experimental diabetic models have been used to investigate neurological disorders associated with diabetes mellitus (DM). Among these models, streptozotocin (STZ)-induced diabetes in rat is unique in that, although there are profound biochemical and

functional abnormalities similar to those in human diabetic neuropathies, morphological changes in the peripheral nerves are mild. This model rat is generally called Streptozotocin (STZ)-induced diabetic rat. Even after extended periods, peripheral nerves fail to show fiber loss or pronounced nerve fiber degeneration (Sima and Sugimoto, 1999). The morphological changes exhibited by rats are a reduction in fiber size with a proximo-distal gradient (Medori et al., 1988; Yagihashi et al., 1990b). Teased fiber examinations have shown nodal swelling, paranodal and segmental demyelination in a small percentage of fibers in rats diabetic for 8-12 months (Sima et al., 1988; Yagihashi et al., 1990a,b). These morphological features closely resemble those seen in entrapment neuropathy. On the other hand, within three months of disease onset, STZ-induced diabetic rats become susceptible to tarsal tunnel syndrome, which is responsive to surgical decompression (Dellon et al., 1994; Kale et al., 2003). In addition, using STZ-induced diabetic rats, Zochodne demonstrated that both excess weight and STZ-induced DM are factors that accelerate tarsal tunnel syndrome (Zochodne et al., 1995). Collectively, STZ-induced diabetic rats appears to be an appropriate model to study the pathological mechanism of entrapment neuropathy associated with DM.

Mast cells which have been shown to play a crucial role in vascular changes, retinopathy, and nephropathy associated with DM may be involved in Schwann cell dysfunction (Ruger et al., 1996; Gilbert et al., 2000). Forcier and others demonstrated in the rat that hyperglycemia increases numbers and degranulation of endoneurial mast cells in a dose dependent manner, which in turn induces Schwann cell injury and demyelination due to hyperosmotic imbalance resulting from polyol pathway activation (Forcier et al., 1991; Mizisin and Powell, 1993). Another possible factor responsible for complications of diabetes is Tenascin-C (TN-C). It has been well established that TN-C is overexpressed by retinal endothelial cells and is deeply involved in diabetic retinopathy by inducing neovascularization (Castellon et al., 2002).

The purpose of this study was to determine the pathomechanism of entrapment neuropathy associated with DM, with special emphasis on the roles of mast cells and TN-C using STZ-induced diabetic rats reported by Zochodne (Zochodne et al., 1995).

Materials and methods

Animals

The white-spotting(Ws)locus of rats represents a 12-base deletion of the c-kit receptor tyrosine kinase. Homozygous Ws/Ws$^{-/-}$ rats are deficient in melanocytes, mast cell, and erythrocytes. The origin procedure of Ws/Ws$^{-/-}$ rats has been described in detail (Niwa et al., 1991). Twenty male Ws/Ws$^{-/-}$ rats (Slc: WsRC-Ws/Ws, Shizuoka, Japan) and 20 of their male wild-type $^{+/+}$ counterparts were used in this study, with the approval of the committee of animal research of Mie University. They were 12 to 16 weeks old, 250 to 300 g. The black rats are their wild-type $^{+/+}$ counterparts and the white are Ws/Ws$^{-/-}$ rats. The bigger rats are nondiabetic rats and smaller are diabetic rats (Fig. 1).

The animals were housed in a temperature-controlled environment and maintained on a 12 h light-dark cycle with food and water available ad libitum. Rats were assigned randomly to the following three groups; diabetic rats raised on wire grid flooring (n=10 in each phenotype), nondiabetic rats raised on wire grid flooring (n=5 in each phenotype), and nondiabetic rats raised on sawdust covered plastic flooring (n=5 in each phenotype) according to Zochodne (Zochodne et al., 1995). Diabetes was initiated in fasting rats by a single intravenous injection of STZ (60 mg/kg, Nakarai Tesque, Kyoto, Japan) in 0.05 M citrate buffer (pH 4.5). Nondiabetic rats received an equivalent volume dosis of the citrate buffered solution. Hyperglycemia was verified 2 weeks after injection by sampling from a tail vein. Whole-blood glucose tests were carried out using ONE TOUCH Ultra (Johnson & Johnson, USA) by cutting the top of the caudal vein. The fasting blood glucose level of 350 mg/dl or over was our criterion for experimental diabetes.

Electrophysiological testing

Fourteen weeks after injection, the sciatic nerves were exposed under anesthesia with 25 mg/kg intraperitoneal pentobarbital (Dainippon Pharma Co, Ltd, Osaka, Japan) and electrophysiological recordings were made. For sciatic-tibial studies, we recorded from the dorsal subcutaneous space of the hind foot using platinum electrodes and stimulated at a point 7.5 cm proximal to the recording point. Distal motor latencies were taken to the onset of the first negative peak from the stimulation. During the recordings, near nerve temperature of 37°C was maintained using a heat lamp. The differences between groups were compared in pairs with Mann-Whitney U test.

Immunohistochemistry

After electrophysiological testing, animals were sacrificed and ankles were harvested bilaterally extending from the proximal tarsal tunnel to the exit of the tarsal tunnel. In addition, a 3-mm segment of the leg was harvested at the middle of the lower leg. Specimens were fixed in 10% formalin and embedded in paraffin. The specimens were cut into 5 μm-thick sections and stained with hematoxylin and eosin (HE). Immunohistochemical studies were performed with monoclonal mouse anti-TN-C antibody (MBL, Nagoya, Japan). The specimens were dewaxed in xylane and rehydrated in graded (99% to 70% (v/v)) methanol in distilled water. Protein Block Serum Free® (DAKO Japan) was applied to sections (15 min, 25°C) to block non-specific binding sites. Immunohistochemical staining was performed using NexES IHC (VENTANA

Japan, Yokohama, Japan) with the monoclonal mouse anti-TN-C antibody (1:100) and the anti-α-SMA antibody (Dako, A/S, Denmark).

Immunofluorescence

After dewaxing and blocking non-binding sites, the sections were treated with primary antibodies for 1 h at room temperature. The polyclonal rabbit anti-laminin antibody (Dako, A/S, Denmark) and the monoclonal mouse anti-TN-C antibody were used. After rinsing 5 times with PBS for 3 min, the specimens were incubated with Alexa Fluor 488 or 546 goat anti-rabbit or anti-mouse IgG antibodies (Molecular Probes, Eugene, OR, USA) for 3 h. Then, they were rinsed and coverslipped with VECTASHIELD (Vector, CA, USA). All slides were examined by fluorescence microscopy (Olympus, BX50, Tokyo, Japan) or confocal laser scanning fluorescence microscopy (Olympus, Fluoview FV1000, Tokyo, Japan) fitted with Argon 488nm and Helium Neon 543nm lasers, which were used to collect three-dimensional (3D) image sets. The digital images from confocal microscopy and some volume images from confocal 3D data sets were analyzed and processed with FV10-ASW software (Olympus, Tokyo, Japan). The final figures were composed using Adobe Photoshop 7.0.

Morphometric analysis

Specimens were viewed using a BX50 microscope (Olympus, Tokyo, Japan) equipped with a video camera. Digitized images were provided on the screen of a computer and morphometric analysis was performed using Lumina Vision (version 1.11) software for Windows (Mitani Shoji Co., Fukui, Japan). To quantitatively evaluate TN-C expression in nerve fascicles, digital images were obtained at x200 magnification and percentage of the specimen area expressing TN-C was calculated as the sum total of the stained areas in each nerve fascicle divided by total area of the fascicle. To quantify perineurial thickening, TN-C expression area in the endoneurium was divided by the endoneurium and perineurium area of the nerve. We analyzed the average of calculated TN-C expression area in perineurium three times.

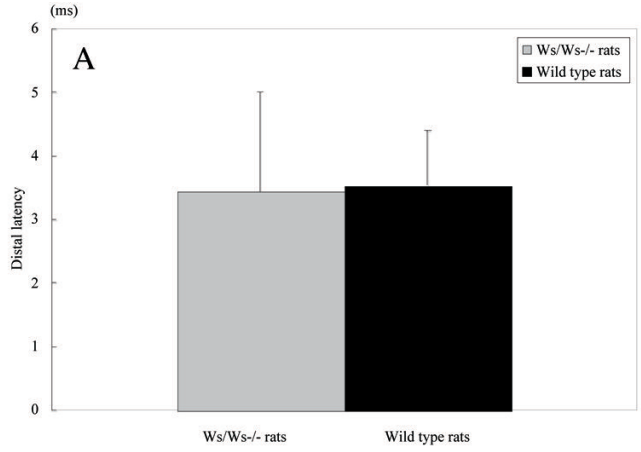
Statistical analysis

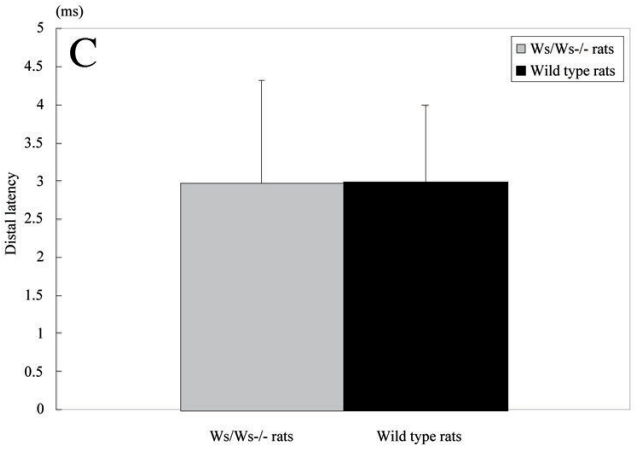
StatView 5.0 for Windows software (SAS Institute, NC, USA) was used for statistical analysis. Data were analyzed using the Mann-Whitely U-test or by calculating Spearman's coefficient of rank correlation.

Results

Diabetes was successfully induced in the animals in this study. The mean weight of the diabetic rats at the conclusion of the study was 220 g, while the mean weight of the normal, nondiabetic rats was 388 g. Four $Ws/Ws^{-/-}$ rats in the diabetic group died 10-12 weeks after injection of STZ. These rats were very emaciated,

Fig. 1. The Black and bigger rat is $^{+/+}$ Non-DM. The black and smaller rat is $^{+/+}$ DM. The white and bigger rat is $Ws/Ws^{-/-}$ Non-DM. The white and smaller rat is $Ws/Ws^{-/-}$ DM.





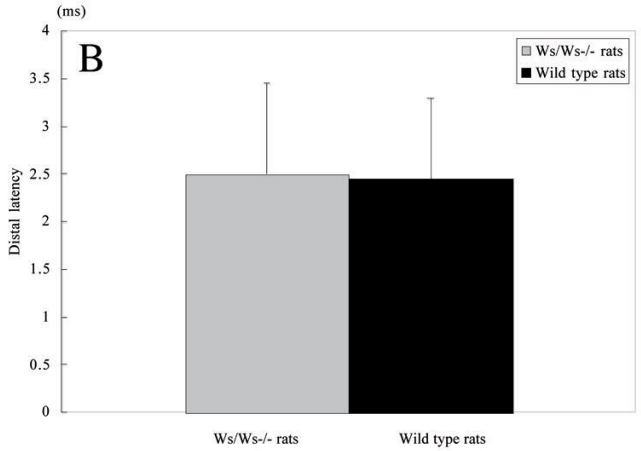


Fig. 2. A. There was no significant correlation with the comparison of 5 Ws/Ws$^{-/-}$ rats with their 5 wild type rats about each distal latency against the background of the diabetic group raised on wire grid flooring. **B.** There was no significant correlation with the comparison of 5 Ws/Ws$^{-/-}$ with their 5 wild-type rats about each distal latency against the background of the nondiabetic group raised on wire grid flooring. **C.** There was no significant correlation with the comparison of 10 Ws/Ws$^{-/-}$ rats with 10 wild type rats about each distal latency against the background of the group raised on wire grid flooring.

but we could not find the cause of their death by dissection. Table 1 showed the mean ± SEM weight and glucose. Diabetic rats were significantly smaller compared with nondiabetic rats.

Mast cells do not play significant roles in entrapment neuropathy associated with DM

Mast cells have been shown to play crucial roles in vasculitis, retinopathy, and nephropathy associated with DM and may have a role in Schwann cell dysfunction (Ruger et al., 1996; Gilbert et al., 2000). In order to test our hypothesis that mast cells could also be involved in entrapment neuropathy, we conducted animal experiments using Ws/Ws$^{-/-}$ rats. First, we compared distal latency in Ws/Ws$^{-/-}$ rats (n=5) with their wild-type counterparts (n=5) raised on wire grid flooring. As shown in Fig. 2A, there was no significant correlation.

Similarly, a comparison of distal latency in nondiabetic Ws/Ws$^{-/-}$ (n=5) and nondiabetic wild-type (n=5) rats raised on wire grid flooring also showed no significant correlation (Fig. 2B). Additionally, we compared distal latency in Ws/Ws$^{-/-}$ (n=10) and wild-type (n=10) rats in all rats-diabetic and nondiabetic-raised on wire grid flooring. Once again, there was no significant correlation between genotype and distal latency (Fig. 2C). These results clearly demonstrate that mast cells do not play a significant role in entrapment

Table 1. Table 1 showed the mean ± SEM weight and glucose 14 weeks after injection of STZ.

rats	weight	glucose mg/
Nondiabetic rats	388±32.3	117±29.2
Diabetic rats	221±27.3	518±72.1

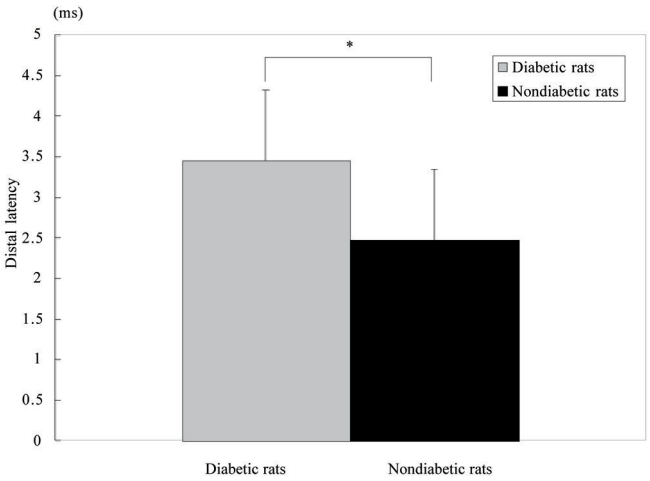


Fig. 3. The distal latency was significantly prolonged in diabetic rats compared with nondiabetic rats at the p<0.01 level.

neuropathy associated with DM.

Not type of flooring or excess weight but hyperglycemia induces entrapment neuropathy

Whole-blood glucose tests were carried out using ONE TOUCH Ultra (Johnson & Johnson, USA) by cutting the top of the caudal vein just before the rats were killed.

Since mast cells did not play a significant role in the development of entrapment neuropathy, the results of Ws/Ws$^{-/-}$ rats and those of their wild-type counterparts were combined in the following statistical analyses. We first compared distal latency in diabetic rats (n=10) and nondiabetic rats (n=10) in groups raised on wire grid flooring. The distal latency was significantly prolonged in diabetic rats compared with nondiabetic rats at the $P<0.01$ level (Fig. 3). Furthermore, blood glucose levels were found to positively correlate ($P<0.05$) with distal latency when comparing diabetic and nondiabetic groups raised on wire grid flooring, suggesting that hyperglycemia may induce entrapment neuropathy associated with DM (Fig. 4).

Next, we studied the effect of chronic mechanical stress on the development of entrapment neuropathy by comparing rats raised on the wire grid flooring and those on the sawdust covered plastic flooring. To test the effect of flooring, we compared distal latency in nondiabetic rats (n=10) raised on wire grid flooring to nondiabetic rats raised on sawdust covered plastic flooring (n=10). As appreciated from Figure 5, the type of flooring did not have any significant effect on distal latency, contrary to Zochondne's results (Zochodne et al., 1995).

Tenascin C expression by the endoneurium may play a significant role in entrapment neuropathy

In the normal nerve, TN-C is expressed only at capillary vessels and the perineurium. In order to analyze the role of TN-C in entrapment neuropathy, we harvested samples in three different areas; proximal to the tarsal tunnel, at the middle of the tarsal tunnel, and at the exit of the tarsal tunnel. Immunohistochemical staining was performed using NexES IHC with anti-TN-C antibody (1:100). In the diabetic group raised on wire grid flooring, TN-C is diffusely overexpressed by both the endoneurium and perineurium at the middle and the exit of the tarsal tunnel (Fig. 6A,B). In contrast, TN-C expression in nerve segments proximal to the tarsal tunnel display a pattern similar to normal nerves (Fig. 6C). This indicates that overexpression of TN-C is a focal phenomenon at the tarsal tunnel.

In contrast to diabetic rats raised on the wire grid flooring, nerves from nondiabetic rats raised on sawdust covered plastic flooring showed normal TN-C expression pattern at all three levels (Fig. 6D).

To further investigate the relationship between abnormal expression of TN-C and entrapment neuropathy, we performed statistical analyses, which demonstrated a significant correlation between TN-C expression on the endoneurium and distal latency at the $P<0.05$ level (Fig. 7).

In order to clearly localize the deposition of TN-C, we analyzed the specimens with a confocal fluorescence microscopy. In contrast to Wallerian degenerated nerves in which TN-C is produced by Schwann cells and deposits within the basal lamina (Martini et al., 1990), TN-C deposits outside of the basal lamina in entrapment neuropathy in diabetic rats raised on wire grid flooring. The red is laminin and the green is TN-C (Fig. 8A). It is useful to explain easily that the green TN-C deposits outside of the red basal lamina around nerve fascicle by 3D photo (Fig. 8B). This indicates that endoneurial cells,

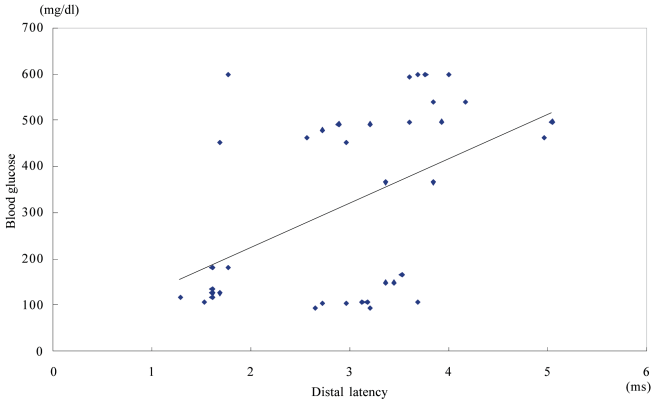


Fig. 4. The blood glucose level was positively correlated with the distal latency at the $p<0.05$ level.

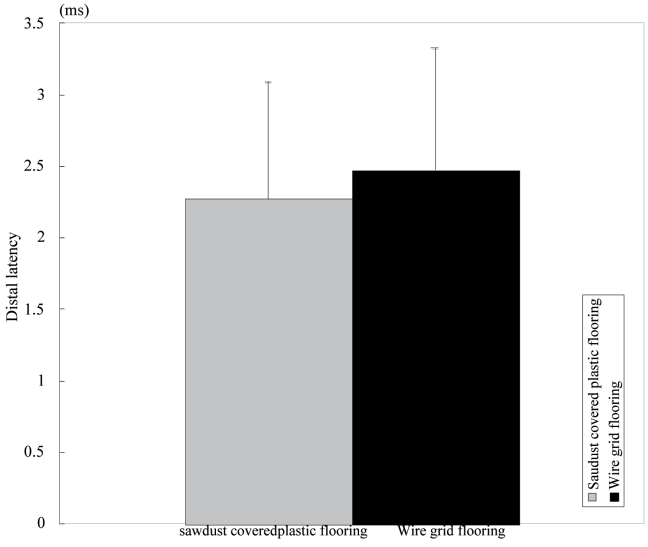


Fig. 5. There was no significant correlation with the comparison of 10 nondiabetic rats with wire grid flooring to 10 nondiabetic rats raised on sawdust covered plastic flooring.

a class of fibroblast outside of the basal lamina, but not Schwann cells, produce the molecule. In order to identify the phenotypes of the fibroblast within the nerve fascicle, we stained the specimens with anti-α-SMA antibody. In normal nerves, no endoneurial cells are positive for α-SMA. In contrast, in nerves from diabetic rats which express TN-C, α-SMA positive myofibroblasts are scattered within the nerve fascicles (Fig. 9). This indicates that hyperglycemia induces phenotypic change of the fibroblast to myofibroblasts.

Thickening of the perineurium may be a protective phenomenon for peripheral nerves

Thickening of the perineurium is a well-known histological characteristic of entrapment neuropathy (Lundborg, 1988). We conducted a careful

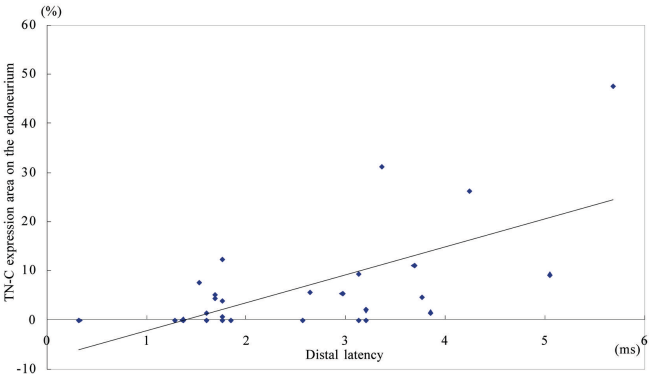


Fig. 7. A significant correlation between TN-C expression on the endoneurium and distal latency at the p<0.05 level was demonstrated.

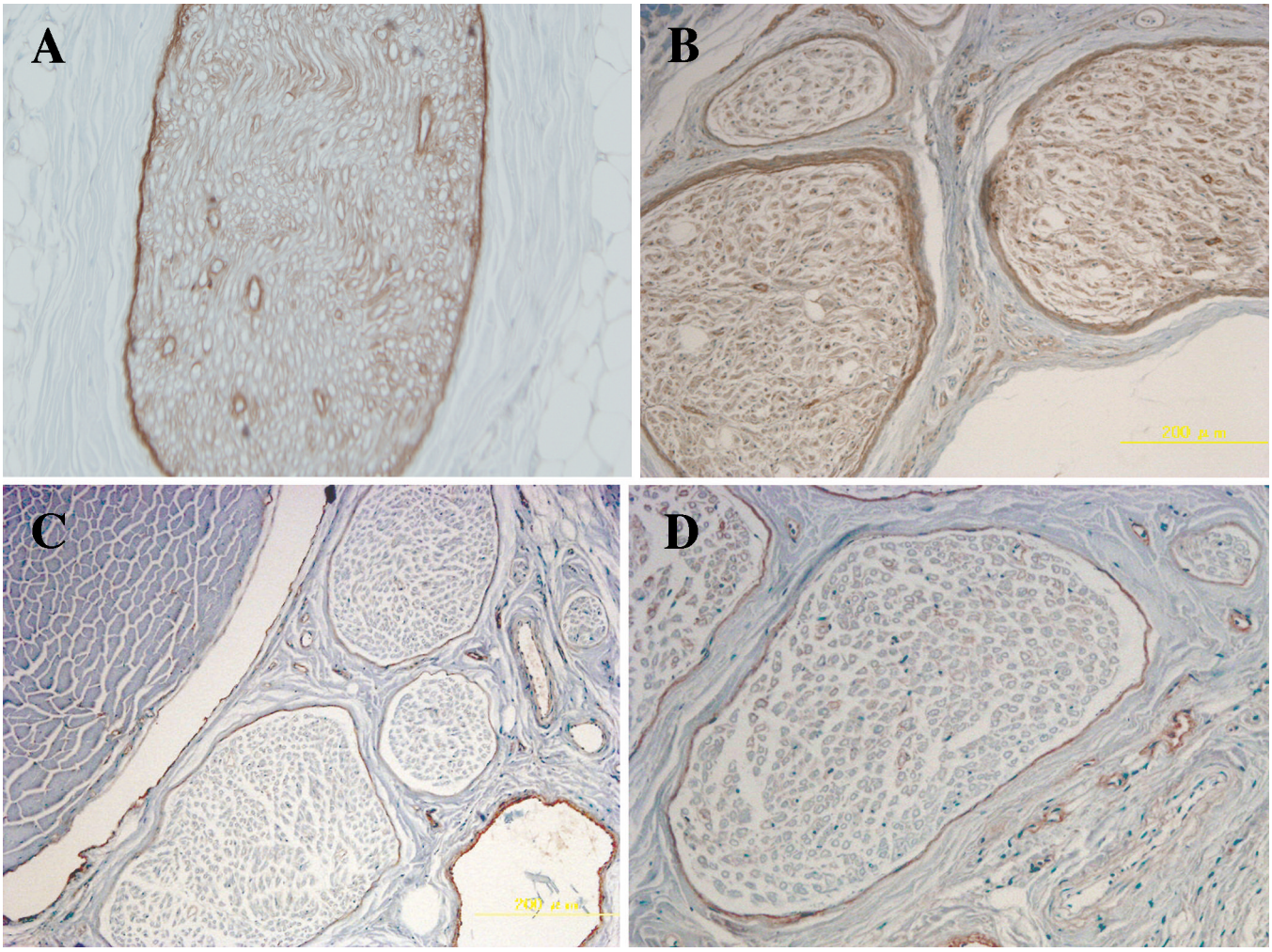


Fig. 6. A. TN-C is diffusely overexpressed by both the endoneurium and perineurium at the middle of tarsal tunnel. x 200. **B.** TN-C is diffusely overexpressed by both the endoneurium and perineurium at the exit of tarsal tunnel. x 100. **C.** TN-C expression in nerve segments proximal to the tarsal tunnel display a pattern similar to normal nerves. x 100. **D.** Normal TN-C expression was shown at the tarsal tunnel in nondiabetic rats raised on sawdust covered plastic flooring. x 200

morphometric analysis of the perineurium to determine whether thickening of the perineurium was correlated with distal latency because there was thickening of the perineurium in some rats (Fig. 10A,B). We compared thickness of the perineurium among three groups; diabetic rats raised on wire grid flooring, nondiabetic rats raised on wire grid flooring, and nondiabetic rats raised on sawdust covered plastic flooring. In contrast to our hypothesis, thickness of the perineurium was highest in nondiabetic rats raised on wire grid flooring followed by diabetic rats raised on wire grid flooring, and was lowest in nondiabetic rats raised on sawdust covered

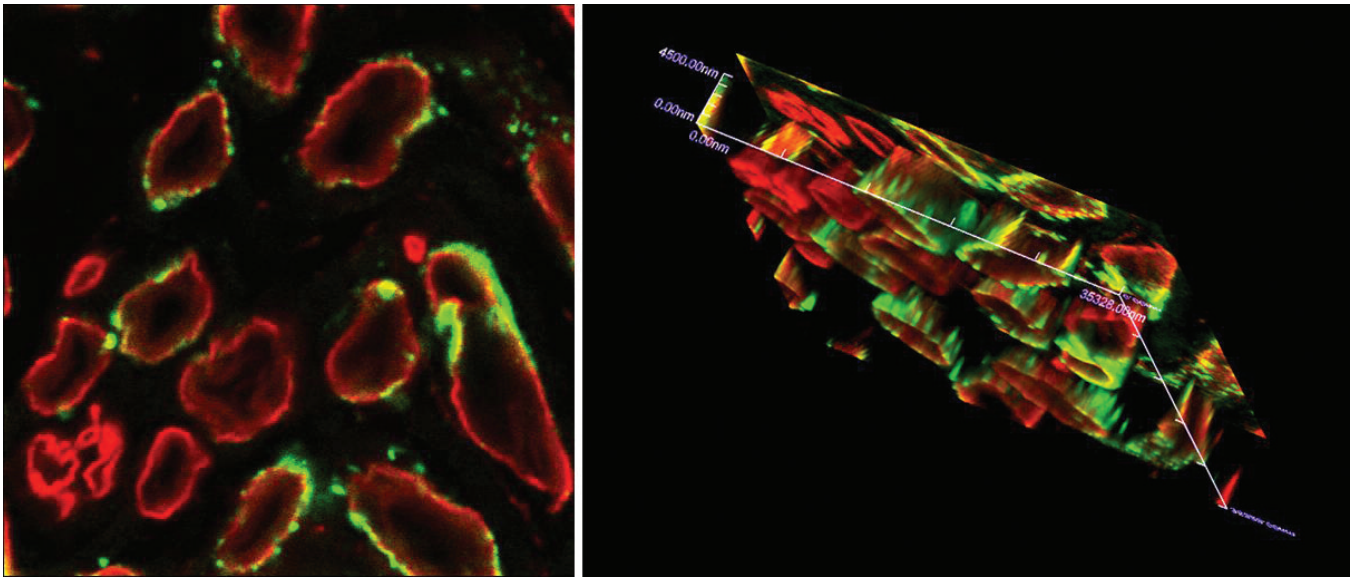


Fig. 8. A. The red is laminin and the green is TN-C. TN-C deposited outside of the basal lamina. **B.** TN-C deposited outside of the basal lamina around nerve fascicle in the 3D image.

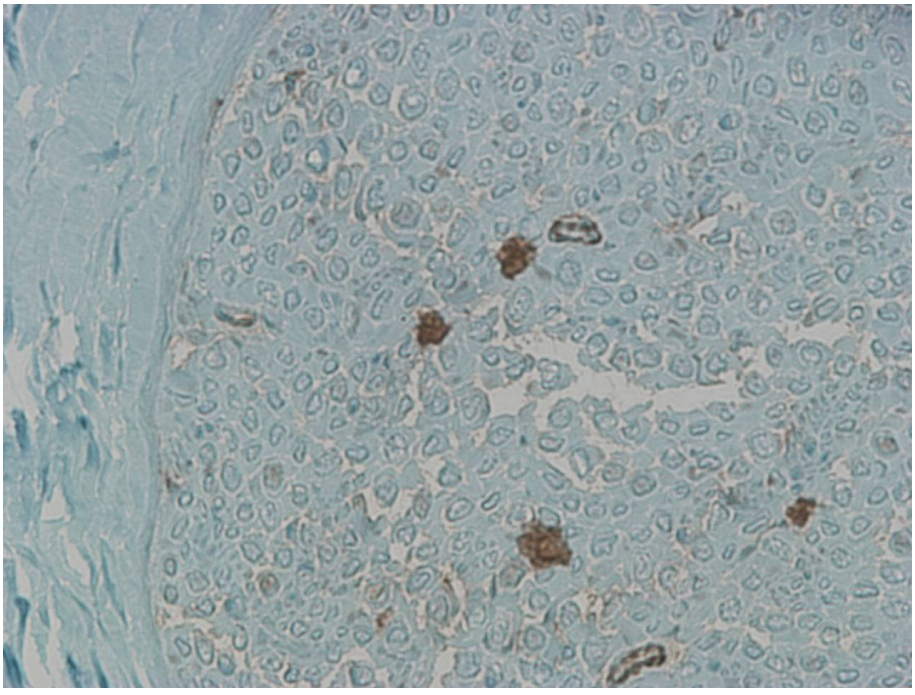


Fig. 9. In nerves from diabetic rats which express TN-C, αSMA positive myofibroblasts are scattered within the nerve fascicles. x 400

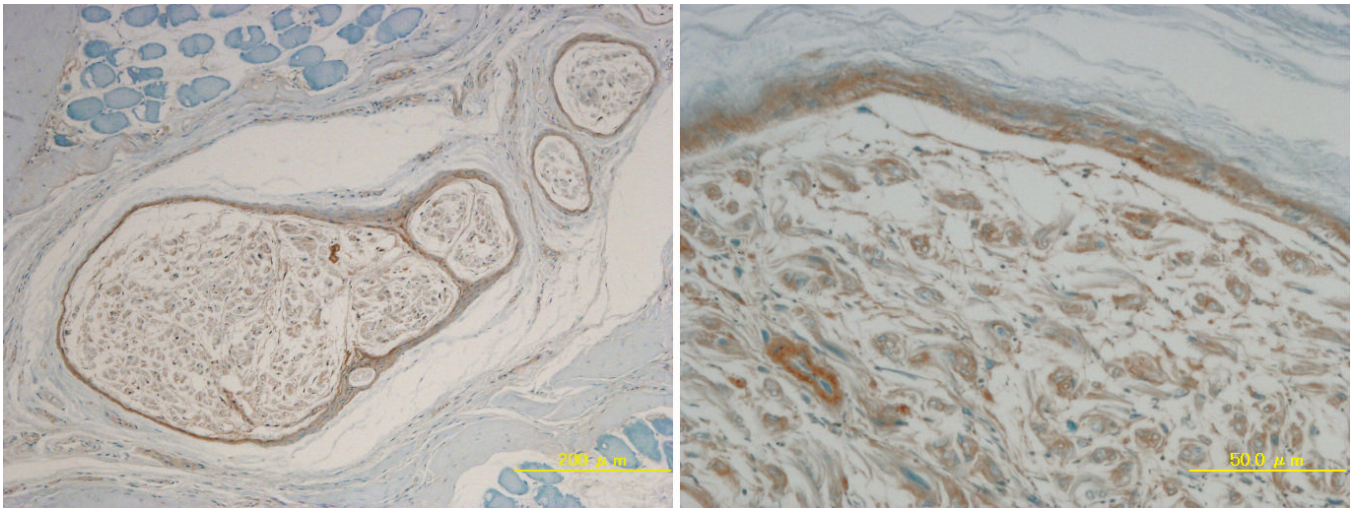


Fig. 10. A. There was thickening of the perineurium at the exit of the tarsal tunnel. x 10. **B.** The perineurium is clearly thick. x 400

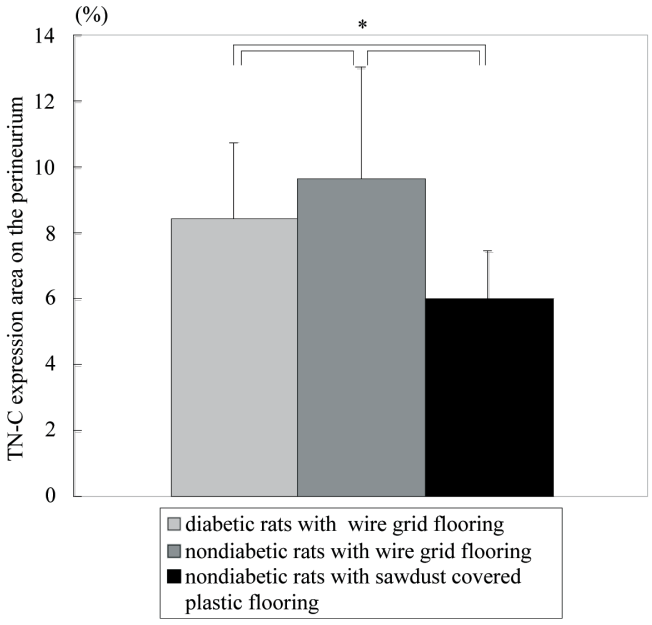


Fig. 11. TN-C expression decreased in the rank order of this graph at the p<0.01 level.

plastic flooring at the P<0.01 level (Fig. 11).

Considering the fact that nondiabetic rats raised on wire grid flooring did not show any significant delay in terminal latency, thickening of the perineurium does not seem to accelerate entrapment neuropathy. On the contrary, thickening of the perineurium seems to be a phenomenon to protect axons.

Discussion

Intensive investigation into diffuse polyneuropathy over the last couple of decades revealed a number of abnormalities, including excessive polyol flux through the aldose reductase pathway, functional and structural alterations of nerve microvessels, nerve and ganglia hypoxia, oxidative stress, nonspecific glycosylation of axons and microvessel proteins, and impairment in the elaboration of trophic factors critical for peripheral nerves and ganglia, which appear to cascade into a 'vicious cycle of progressive microvascular disease associated with motor, sensory, and autonomic fiber loss' (Yagihashi, 1995; Sima and Sugimoto, 1999). In contrast, although the entrapment neuropathies are highly prevalent in the diabetic population, with the estimation that one in every three patients has one, little is known about why patients with DM are predisposed to entrapment neuropathies (Vinik and Mehrabyan, 2004).

Zochodne investigated the influence of DM on the development of entrapment neuropathy using the same model as we used in this study and concluded that DM merely accelerates the entrapment neuropathy (Zochodne et al., 1995). In contrast to Zochodne's results, excess weight and grid flooring alone did not significantly reduce either amplitude or terminal latency in the present study. This may be due to the lower body weight of the rats and shorter disease duration as compared to Zochodne's study, however, combination of DM and grid flooring did cause tarsal tunnel syndrome.

The pathomechanism of entrapment neuropathy is generally believed to be regional chronic compression of nerves (Lundborg, 1988). Previous histological evaluations using various chronic nerve compression models have revealed that demyelination occurs in the periphery as early as 4 weeks of compression. With longer periods of compression, epineurial and perineurial fibrosis occurs and nerve fiber pathology develops. A "neural scar" forms secondary to fibroblast proliferation. Axonal integrity is preserved until relatively late in the process, but eventually axonal

numbers and function diminish (Gupta et al., 2004). However, it is still unclear why demyelination occurs and how fibroblast proliferation is induced by sustained chronic compression.

In the present study, we investigated the pathological events in the nerve trunk using naturally developing tarsal tunnel syndrome model with special emphasis on the roles of mast cells and TN-C. Endoneurial mast cells have been found to play significant roles both in physiological and pathological conditions (Skaper et al., 2001; Esposito et al., 2002). Based on the reports that hyperglycemia increases numbers and degranulation of endoneurial mast cells and inhibition of aldose reductase ameliorates Schwann cell dysfunction in DM (Forcier et al., 1991), we hypothesized that mast cells are involved in the vulnerability of DM patients to entrapment neuropathies. However, contrary to our expectations, there was not any significant difference between $Ws/Ws^{-/-}$ rats and their wild-type counterparts either in electrophysiological tests or upon histological evaluation. Therefore, it seems unlikely that mast cells make a significant contribution to the onset or progression of either idiopathic or DM-associated entrapment neuropathies. On the other hand, TN-C seems to play a significant role in the development of entrapment neuropathy. Although TN-C is highly and diffusely expressed along peripheral nerves during neurogenesis, only the perineurium continues to express the molecule in peripheral nerves after birth (Garcion et al., 2001). However, upon axonal injury, Schwann cells de-differentiate from a myelinated to an unmyelinated phenotype and start to express TN-C at high levels (Martini et al., 1990). Therefore, we speculated that TN-C was deposited in nerve fascicles by Schwann cells. However, confocal fluorescence microscopy clearly demonstrated that TN-C deposits outside of the basal lamina, indicating that cells outside of the basal lamina rather than Schwann cells produce TN-C. It is well known that TN-C is expressed during wound healing in various tissues. For example, we recently demonstrated that TN-C is highly expressed by the myofibroblast during the acute stage of myocardial infarction (Tamaoki et al., 2005). It stimulates cell migration, smooth muscle actin expression, and collagen contraction. In addition, TN-C gene expression was recently found to be mechanosensitive (Chiquet-Ehrismann et al., 1994). We carried out an in vitro study with cultured tenosynovial fibroblasts from patients with CTS and a vacuum-operated stress-providing instrument that applies cyclic tension to cells. These experiments clearly demonstrated that mechanical stress significantly up-regulates TN-C production by fibroblasts (Tsujii et al., 2006). Taken together, we think that chronic compression applied to the nerve induces TN-C expression by the endoneurial and perineurial cells, which in turn stimulates thickening of the perineurium and intraneural fibrosis. It should be noted that nerves with diffuse and intense TN-C expression by the endoneurium contained a specific class of fibroblast, i.e. myofibroblast. Myofibroblasts have been shown to play significant roles in wound healing by producing a number of extracellular matrix proteins including TN-C. In addition, myofibroblasts strongly induce collagen contraction (Tamaoki et al., 2005). Therefore, we speculate that in nerves expressing TN-C within the endoneurium, the endoneurium constrict axons.

In contrast to TN-C expression by the endoneurium, thickness of TN-C expressing perineurium was significantly greater in the nondiabetic group than in the diabetic group, suggesting, contrary to the accepted notion, that perineurial thickening is not an accelerating factor but appears to be a protective phenomenon for entrapment neuropathy. Malfunction of the perineurial cells from diabetes thus appears to make the axons more liable to compression neuropathy.

In conclusion, the present study demonstrated that DM is a significant predisposing factor for entrapment neuropathies and that TN-C expression in the endoneurium is closely correlated with nerve function. Considering the fact that nondiabetic rats exposed to more mechanical stress from grid flooring than diabetic rats due to their heavier weight, it seems likely that metabolic and phenotypic abnormalities of endoneurial and perineurial fibroblasts lies behind the vulnerability of DM patients to entrapment neuropathy. In contrast to angiopathies, retinopathy, and nephropathy, three representative complications of DM, mast cells do not play significant roles in the onset or progression of the entrapment neuropathy associated with DM.

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