Pathology of acute and chronic ischaemic neuropathy in atherosclerotic peripheral vascular disease

Hitoshi Nukada,¹ Andre M van Rij,² Stephen G. K. Packer² and P. Denise McMorran¹

Departments of ¹Medicine and ²Surgery, University of Otago Medical School, Dunedin, New Zealand

Correspondence to: H. Nukada, Department of Medicine, University of Otago Medical School, PO Box 913, Great King Street, Dunedin, New Zealand

Summary
The peripheral nerve pathology in ischaemic limbs with atherosclerotic peripheral vascular diseases (PVD) is difficult to ascertain because of the limited number of reports. In addition, it has been debated whether chronic ischaemia per se could cause morphological abnormalities in peripheral nerves. In this prospective study, we examined pathological findings in the sural, saphenous, deep peroneal, superficial peroneal and tibial nerves, taken from seven acutely and nine chronically ischaemic amputated legs in which ischaemia was due to non-diabetic severe PVD. For morphological comparison, nerves were also taken from amputated legs without ischaemic disease and those in which PVD was associated with diabetes. In acutely ischaemic nerves, pathological changes were dependent upon the duration of ischaemia. Axonal degeneration of both myelinated and unmyelinated nerve fibres (MFs and UMFs) with occluded vessels was prominent, if acute ischaemia was present for >24 h. Focal lesions, a hallmark of acute ischaemic neuropathy, were seen in both acute and chronic PVD nerves. Chronic PVD nerves also revealed considerable variations in the density of MFs between the fascicles of individual nerves and between the nerves of individual subjects; demyelination and remyelination, endoneurial oedema particularly at the subperineurial region, swollen endothelial cells, various but infrequent axonal changes, and relative preservation of UMFs were also seen. All pathological changes found in acute and chronic PVD nerves, except for a high rate of demyelinated and remyelinated nerve fibres, have been described in experimental models of acute ischaemic/reperfusion injury. Demyelination could be induced by chronic ischaemia. Thus, pathological alterations in chronic ischaemic neuropathy may be due to the combined effects of acute ischaemia/reperfusion and chronic hypoxia.

Keywords: vascular disease; ischaemic neuropathology; ischaemic limb; nerve pathology

Abbreviations: MF = myelinated nerve fibre; PVD = peripheral vascular disease; UMF = unmyelinated nerve fibre

Introduction
The term ‘ischaemic neuritis’ or ‘ischaemic neuropathy’ is used to describe peripheral nerve lesions associated with and caused by atherosclerotic occlusive PVD, which is a common problem among the elderly (Hutchinson, 1970). The recent Edinburgh Artery Study reported that, in addition to 4.5% of the general population aged between 55 and 74 having intermittent claudication, almost twice that number (8.0%) had abnormal vascular laboratory tests (Ruckley, 1991). Although neuropathic abnormalities are often seen in ischaemic limbs with PVD, Asbury (1970) stated that ‘perhaps the most salient feature of this particular type of peripheral nerve disorder resides in the fact that it is almost always overlooked because of more obvious effects of peripheral vascular insufficiency upon skin and muscle’. The peripheral nerve pathology with PVD is difficult to ascertain because of incomplete data (Daube and Dyck, 1984; Chalk and Dyck, 1993). It is also uncertain whether chronic ischaemia, insufficient to cause infarction, could induce structural changes in peripheral nerves.

Mufson (1952) demonstrated that 37% of 145 PVD subjects had neuropathic impairment in ischaemic legs. Sensory symptoms and signs were the most common findings, but reflex changes and muscle weakness were also noted. Hutchinson and Liversedge (1956) suggested that the presence
of neuropathy was directly related to the severity of PVD, although the most striking feature was the mildness of the neurological abnormalities in spite of advanced PVD. The highest frequency of neuropathic deficit in PVD was reported by Eames and Lange (1967) who found such abnormalities in 88% of 32 subjects with severely ischaemic limbs, although some neurological findings may be part of the general ageing process. A high frequency of sensory dominant neuropathic signs (74%) in ischaemic limbs was also reported by Chopra and Hurwitz (1969a). Abnormalities in nerve conduction and EMG have been well recognized in chronic PVD subjects (Miglietta and Lowenthal, 1962; Miglietta, 1966; Chopra and Hurwitz, 1969a; D'Amour et al., 1987; Hunter et al., 1988; England et al., 1992, 1995) and in acute PVD (Lachance and Daube, 1991). These abnormalities include slowed conduction velocity and low amplitude in common peroneal, posterior tibial and sural nerves, and evidence of distal denervation.

In spite of relatively few pathological studies in PVD nerves, various morphological abnormalities have been described such as segmental demyelination and remyelination, axonal degeneration and a loss of nerve fibres. Segmental demyelination is probably the most common feature, while axonal degeneration is dominant in some cases. Previous reports on nerve pathology in PVD subjects are summarized in Table 1. Priestley (1931, 1932) was the first to describe morphological changes of peripheral nerves in amputated ischaemic legs with non-diabetic PVD in the English literature. The extent of nerve pathology was directly proportional to the degree of pathologically observed arteriosclerotic changes in vessels. In cases of thromboangiitis obliterans, patchy nerve fibre degeneration involving certain fascicle or fibres was observed in peroneal and tibial nerves of 17 amputated legs (Barker, 1938).

With the development of a single teased fibre technique and electron-microscopic analysis, evidence of a considerable amount of segmental demyelination and remyelination became apparent in chronic PVD nerves (Chopra and Hurwitz, 1967; Eames and Lange, 1967). Eames and Lange (1967) also observed (i) a certain amount of axonal degeneration, (ii) that most of unmethylated nerve fibres (UMFs) appeared normal, (iii) basement membrane thickening and proliferated endothelial cells in endoneurial capillaries, and (iv) thrombosed epineurial arterioles. In contrast to the previous studies, Chopra and Hurwitz (1969b) reported no obvious loss of myelinated nerve fibres (MFs) in chronic PVD nerves. Farinon et al. (1984) found varying severity of pathological changes, although four out of seven subjects were diabetic. Similar heterogeneous pathology in type and severity in chronic PVD nerves was described by Vital et al. (1986). In superficial peroneal nerves, they demonstrated that (i) the loss of MFs varied from case to case, (ii) axonal degeneration was prevalent, (iii) segmental demyelination was not prominent, and (iv) the severity of UMF involvement differed among cases. These results suggested that various pathological abnormalities could be found in chronic PVD nerve, although the factors affecting the variability of nerve pathology remain uncertain.

Aims of this prospective study were to demonstrate morphological changes among different peripheral nerves in severe acute and chronic PVD subjects, and to compare the pathological finding in acute PVD nerves with those in chronic PVD. We examined pathological features in sural, saphenous, deep peroneal, superficial peroneal, and tibial nerves, taken from acutely and chronically ischaemic amputated limbs due to non-diabetic PVD. Nerve pathology in these PVD legs was compared with those in non-ischaemic legs and in legs in which ischaemia was due to chronic PVD associated with diabetes. All nerve specimens were obtained from amputated legs.

Material and methods

Subjects

Twenty-two subjects, 14 males and eight females admitted to the Surgery Department, Dunedin Hospital for below knee amputation between 1989 and 1993 were studied. Seven (mean age, 67.0 years) suffered from severe acute ischaemia resulting from non-diabetic atherosclerotic occlusive disease (hereafter ‘acute PVD’), and nine (mean age, 72.3 years) had threatened ischaemic limb due to severe chronic non-diabetic atherosclerotic occlusive disease (‘chronic PVD’). Clinical and laboratory data in acute and chronic PVD subjects are summarized in Tables 2 and 3. Three acute PVDs were preceded by the surgery of abdominal aorta aneurysm (Cases 1, 2 and 4), and four other had embolism (Cases 3, 5, 6 and 7). None of the acute PVD subjects had a history of intermittent claudication. In acute PVD subjects, there was no signs of peripheral nerve involvement except one (Case 7) who showed mild sensory impairment up to ankle in the leg to be amputated. Control nerves were obtained from three subjects of a similar age range suffering from sepsis (age: mean 66.3 years, range 62–71 years; hereafter ‘non-ischaemic control’). None of these control subjects had a history of heavy alcoholic intake, diabetes or any other disease which might cause vasculopathy or peripheral neuropathy. For comparison, nerves in three subjects with chronic PVD associated with diabetes were also taken (age: mean 74.0 years, range 61–83 years; ‘diabetic chronic PVD’). Diabetic chronic PVD subjects had clinical and electrophysiological evidence of severe polyneuropathy associated with ulcers in their legs. Two of them had no rest pain. This study was approved by the Ethics Committee of the Southern Regional Health Authority.

All subjects in chronic PVD presented with complaints of pain at rest associated with skin necrosis or ulcer in their legs to be amputated, and had a history of multiple vascular reconstructive interventions in their ischaemic limbs at some time before the below knee amputation. These surgical procedures include endarterectomy, femoro-iliofemoral thrombectomy, femoro-popliteal bypass and aorto-femoral...
Table 1 Pathological findings of peripheral nerves in non-diabetic peripheral arterial occlusive diseases

<table>
<thead>
<tr>
<th>Authors</th>
<th>Cases/course</th>
<th>Nerves examined*</th>
<th>Major pathological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priestly (1931, 1932)</td>
<td>6 chronic</td>
<td>Posterior tibial nerve at four levels&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Degeneration&lt;sup&gt;2&lt;/sup&gt; and fibrosis, more prominent distally</td>
</tr>
<tr>
<td>Gairns &lt;i&gt;et al.&lt;/i&gt; (1960)</td>
<td>1 chronic</td>
<td>Digital nerve of the great toe</td>
<td>Severe loss of MFs</td>
</tr>
<tr>
<td>Garven &lt;i&gt;et al.&lt;/i&gt; (1962)</td>
<td>2 chronic</td>
<td>Posterior tibial, plantar, and digital nerves at several levels</td>
<td>Progressive distal loss of MFs with marked reduction in digital nerves, vascular swelling</td>
</tr>
<tr>
<td>Eames and Lange (1967)</td>
<td>8 chronic</td>
<td>Sural nerve</td>
<td>Demyelination and remyelination &gt; axonal degeneration, obvious loss of MFs, vascular swelling, preservation of UMFs</td>
</tr>
<tr>
<td>Chopra and Hurwitz (1967, 1969)</td>
<td>6 chronic</td>
<td>Sural nerve</td>
<td>Demyelination and remyelination &gt; axonal degeneration, no loss of MFs</td>
</tr>
<tr>
<td>Farinon &lt;i&gt;et al.&lt;/i&gt; (1984)</td>
<td>7 chronic&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Sciatic and tibial nerves</td>
<td>Demyelination and remyelination and/or axonal degeneration, various loss of MFs, axonal atrophy, preservation of UMFs</td>
</tr>
<tr>
<td>Vital &lt;i&gt;et al.&lt;/i&gt; (1988)</td>
<td>12 chronic</td>
<td>Superficial peroneal nerves</td>
<td>Axonal degeneration &gt; demyelination, various loss of MFs, focal lesion, UMF involvement</td>
</tr>
<tr>
<td>Hunter &lt;i&gt;et al.&lt;/i&gt; (1988)</td>
<td>1 chronic</td>
<td>Posterior tibial and sural nerves</td>
<td>Marked loss of MFs, microvascular occlusion</td>
</tr>
<tr>
<td>Rodriguez-Sanchez &lt;i&gt;et al.&lt;/i&gt; (1991)</td>
<td>8 chronic</td>
<td>Sural nerve</td>
<td>Various types and severities of pathology, demyelination and remyelination or axonal degeneration, UMF abnormalities</td>
</tr>
<tr>
<td>Nukada &lt;i&gt;et al.&lt;/i&gt; (1996)</td>
<td>7 acute 9 chronic</td>
<td>Tibial, sural, saphenous, superficial peroneal and deep peroneal nerves</td>
<td>Acute PVD: focal lesions, axonal degeneration, occluded vessels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chronic PVD: focal and multifocal lesions, various MF densities, remyelination &gt; demyelination &gt; axonal degeneration, endoneurial oedema, vascular swelling, preservation of UMFs</td>
</tr>
</tbody>
</table>

MF = myelinated nerve fibre; UMF = unmyelinated nerve fibre; PVD = peripheral vascular disease. *All nerves were taken at the time of leg amputation, except five nerve biopsy cases by Chopra and Hurwitz. †From the popliteal space to the internal malleolus. ‡The term ‘degeneration’ was applied to nerve fibres with true Wallerian degeneration or fascicle with a definite loss of MFs. §Including four diabetic subjects.

Table 2 Clinical and laboratory data on seven amputated legs with non-diabetic acute PVD

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (years)/sex</th>
<th>Side</th>
<th>Duration*</th>
<th>Rest pain</th>
<th>Aetiology</th>
<th>Site of occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82/F</td>
<td>L</td>
<td>24 h</td>
<td>No</td>
<td>Post-AAA</td>
<td>Superficial femoral</td>
</tr>
<tr>
<td>2</td>
<td>79/M</td>
<td>R</td>
<td>2 days</td>
<td>No</td>
<td>Post-AAA</td>
<td>Superficial femoral</td>
</tr>
<tr>
<td>3</td>
<td>69/F</td>
<td>L</td>
<td>4 days</td>
<td>Yes</td>
<td>Embolism</td>
<td>Superficial femoral</td>
</tr>
<tr>
<td>4</td>
<td>79/M</td>
<td>L and R</td>
<td>5 days</td>
<td>No</td>
<td>Post-AAA</td>
<td>Popliteal and tibial</td>
</tr>
<tr>
<td>5</td>
<td>62/M</td>
<td>R</td>
<td>1 month</td>
<td>Yes</td>
<td>Embolism</td>
<td>External iliac and superficial femoral</td>
</tr>
<tr>
<td>6</td>
<td>55/F</td>
<td>R</td>
<td>1 month</td>
<td>Yes</td>
<td>Embolism</td>
<td>Diffuse, mainly distal small</td>
</tr>
<tr>
<td>7</td>
<td>55/M</td>
<td>R</td>
<td>6 months</td>
<td>Yes</td>
<td>Thrombosis</td>
<td>Superficial femoral and popliteal</td>
</tr>
</tbody>
</table>

AAA = abdominal aorta aneurysm. *Duration after the onset of ischaemia.

bypass graft. All chronic PVD subjects revealed unilateral or asymmetrical sensory impairment to all modalities in the legs to be amputated; at regions of deep peroneal or saphenous nerves in five legs, and stocking distribution up to ankle or lower-calf levels in four. Mild weakness of dorsiflexion of toes and foot (4/5 MRC grading) on the amputated side was observed in three subjects. Nerve conduction studies of common peroneal and sural nerves evaluated prior to amputation in seven chronic PVD subjects showed a variety of mild to moderate abnormal results, e.g. slowed conduction velocity and reduced amplitude (Table 3).

Pathological assessments
Sural, saphenous, deep peroneal, superficial peroneal, and tibial nerves at the lower-calf to ankle levels were taken
Table 3 Clinical and laboratory data on nine amputated legs with non-diabetic chronic PVD

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (years)/sex</th>
<th>Side</th>
<th>Duration*</th>
<th>Rest pain</th>
<th>ABI at rest</th>
<th>Site of occlusion</th>
<th>Sural nerve conduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>83/F</td>
<td>R</td>
<td>6</td>
<td>Yes</td>
<td>0.3</td>
<td>Popliteal</td>
<td>↓NCV†</td>
</tr>
<tr>
<td>9</td>
<td>73/F</td>
<td>R</td>
<td>3</td>
<td>Yes</td>
<td>↓</td>
<td>Popliteal and all vessels in calf</td>
<td>↓Amplitude§</td>
</tr>
<tr>
<td>10</td>
<td>72/M</td>
<td>L</td>
<td>5</td>
<td>Yes</td>
<td>0.40</td>
<td>Superficial femoral</td>
<td>NT</td>
</tr>
<tr>
<td>11</td>
<td>75/M</td>
<td>R</td>
<td>3</td>
<td>Yes</td>
<td>0.42</td>
<td>Superficial femoral</td>
<td>↓Amplitude§ and ↓NCV†</td>
</tr>
<tr>
<td>12</td>
<td>65/M</td>
<td>L</td>
<td>2</td>
<td>Yes</td>
<td>0.28</td>
<td>Superficial femoral and popliteal</td>
<td>↓NCV†</td>
</tr>
<tr>
<td>13</td>
<td>65/F</td>
<td>L</td>
<td>12</td>
<td>Yes</td>
<td>0.22</td>
<td>Internal iliac, superficial femoral and popliteal</td>
<td>NT</td>
</tr>
<tr>
<td>14</td>
<td>75/M</td>
<td>L</td>
<td>1.5</td>
<td>Yes</td>
<td>↓</td>
<td>Popliteal</td>
<td>↓NCV†</td>
</tr>
<tr>
<td>15</td>
<td>64/M</td>
<td>R</td>
<td>3</td>
<td>Yes</td>
<td>↓</td>
<td>Aorta and superficial femoral</td>
<td>↓NCV†</td>
</tr>
<tr>
<td>16</td>
<td>79/M</td>
<td>L</td>
<td>6</td>
<td>Yes</td>
<td>0.14</td>
<td>Superficial femoral</td>
<td>NT</td>
</tr>
</tbody>
</table>

ABI = ankle brachial index; NT = not tested. *History of intermittent claudication; †slowed nerve conduction velocity; ‡unmeasurable; §reduced amplitude.

immediately after amputation. Nerve processing for pathological evaluation has been detailed previously (Nukada et al., 1989). In brief, nerve specimens for plastic sections were fixed with 2.5% glutaraldehyde in 0.025 M cacodylate buffer, pH 7.4, at room temperature for 24 h. Nerves were cut into smaller pieces (1–2 mm in length), then postfixed in 2% osmium tetroxide for 24 h, dehydrated in serial alcohols and propylene oxide, and embedded in Araldite. Transverse sections, 1.0 μm in thickness, were cut and stained with methylene blue, thionin, and acridine orange, and phenylenediamine. Ultra-thin sections were stained with uranyl acetate followed by lead citrate. Nerve segments for teased fibre analysis were fixed for 30 min in glutaraldehyde, and then postfixed in 2% osmium tetroxide for 8 h. A minimum of 100 single fibres were prepared and graded (Dyck et al., 1993). Morphometric measurements of MFs on transverse semi-thin sections were performed directly from slides with the use of a SAMBA 2005 computerized image analysis system (TITN, Grenoble, France) (Wright and Nukada, 1994). The coefficient of variation and the index of dispersion of MF density (mm⁻²) were employed for recognition of variability of MF density within and between fascicles (Dyck et al., 1984). Data were expressed as mean±SD, and P values <0.05 were considered significant using the two-tailed Student’s t test.

Results

Acute PVD nerves

Pathological changes in acutely ischaemic nerves were dependent on the duration of ischaemia. Teased fibres in Case 1 whose nerves were taken after 24 h of ischaemia revealed <5% of MFs with axonal degeneration. Axonal degeneration of both MFs and UMFs was conspicuous if acute ischaemia was present for 48 h. Panfascicular or central fascicular fibre degeneration with occluded vessels was prominent in Cases 2, 3 and 4 (Fig. 1). Nerves taken at 1–6 months after the onset of acute limb ischaemia showed variable MF densities with regenerated fibres (Cases 5, 6 and 7). Endoneurial oedema and MFs with abnormally thin myelin were also noted in these three cases, although less noticeable when compared with those in chronic PVD nerves.

Fig. 1 Transverse section of the superficial peroneal nerve taken from Case 4 who had acute PVD showing panfascicular axonal degeneration with occluded vessels in endo-, peri- and epineurial regions. Plastic-embedded 1 μm thick section, thionin and acridine orange staining. Bar = 100 μm.

Chronic PVD nerves

Focal lesions such as selective damage at central or subperineurial fascicular regions were observed in all tibial nerves and most of the saphenous and superficial peroneal nerves (Figs 2–4). Central fascicular lesions were more prominent in large fascicles than smaller fascicles, whereas subperineurial lesions were observed in both large and small fascicles. These focal lesions were seen either to be due to loss of nerve fibres (Fig. 2) or were filled with regenerating fibres (Fig. 3). The variability of MF numbers was obvious between the fascicles of the individual nerves (Fig. 2), and between the nerves of the individual subjects (Fig. 5). A complete loss of MFs was found only in one saphenous nerve taken from Case 10 (Fig. 5A), while MF
Fig. 2 Pathological appearance of the tibial nerve at the lower-thigh level taken from Case 11, who had chronic PVD, showing the variation of the density of myelinated nerve fibres. A central fascicular lesion is seen in the left fascicle, and the number of myelinated nerve fibres is relatively preserved in the right lower fascicle when compared with other fascicles. Note endoneurial oedema, at the subperineurial region in particular. This tibial nerve contains 21 fascicles. Plastic-embedded 1 μm thick section, methylene blue stain. Bar = 200 μm.

Fig. 3 Central fascicular lesion filled with regenerating nerve fibres in the tibial nerve from Case 10 who had chronic PVD. Note subperineurial oedema and myelinated nerve fibres with vesicular myelin swelling. Plastic-embedded 1 μm thick section, methylene blue stain. Bar = 100 μm.
Fig. 4 Subperineurial lesion associated with subperineurial oedema in the saphenous nerve from Case 12 who had chronic PVD. Plastic-embedded 1 μm thick section, thionin and acridine orange staining. Bar = 30 μm.

densities of other nerves in this subject were only moderately reduced (Fig. 5B).

Nerve fibres with disproportionately thin myelin relative to axon area were commonly found (Fig. 6). In the teased nerve study, the frequency of remyelinated fibres was significantly greater in chronic PVD nerves than in non-ischaemic controls for all the nerves examined except the saphenous nerve (Table 4). Demyelinated nerve fibres were also observed (Fig. 6C) and teased fibre analysis demonstrated that demyelination was more frequently seen in chronic PVD nerves than in controls, but statistically significant difference was reached only in the sural nerve (Table 4). Myelinated nerve fibres with myelin infolding and clefts were often observed particularly in the fascicles with severe reduction in the number of MFs.

Subperineurial oedema was another prominent feature (Figs 2-4), although not observed in all the fascicles evaluated. Nerves, which showed reduced amplitude of nerve action potential electrophysiologically (Table 3), revealed particularly severe endoneurial oedema. Mean transverse fascicular areas of each nerve were not significantly different between chronic PVD nerves and controls (6.1±0.9 mm² and 5.3±1.2 mm² for tibial nerves, respectively). Intramyelinic oedema was also frequently found in chronic PVD nerves (Figs 3 and 5B). Nerve fibres showing axonal changes were less commonly seen, and the teased fibre study confirmed the infrequency of axonal degeneration (Table 4). However, darkly stained swollen axons or attenuated axons were observed in chronic PVD nerves (Fig. 7). Unmyelinated nerve fibres appeared to be normal, even in nerves revealing a definite loss of MFs, except the saphenous nerve, which showed a complete loss of MFs, had degenerated UMFs. Endoneurial vessels exhibited swollen endothelial cells. Results of vascular morphometry will be reported separately.

Mean MF densities (mm⁻²) in chronic PVD nerves were moderately but significantly reduced when compared with those in non-ischaemic control nerves (Table 5). The index of dispersion and the coefficient of variation of MF densities between the sampled frames within the fascicle and between the fascicles within the same nerve were significantly greater in chronic PVD nerves than in non-ischaemic controls: for tibial nerves, mean coefficient of variation and index of dispersion of MF density among the frames was 45.5±10.6% in chronic PVD and 30.6±6.2 in controls (P < 0.01) and 2.5±0.7 in chronic PVD and 1.4±0.5 in controls (P < 0.02), respectively; and similar trends were noted for the coefficient of variation and the index of dispersion of MF density among the fascicles.

**Control nerves**

**Non-ischaemic controls**

Neither focal nor multifocal pathology was seen. MFs with disproportionately thin myelin were seen and teased fibre study demonstrated that ~20–30% of MFs revealed remyelination, presumably due to part of the general ageing process in peripheral nerve. Axonal degeneration or nerve oedema was not commonly found.

**Diabetic chronic PVD**

Nerves in diabetic chronic PVD exhibited a marked diffuse loss of nerve fibres in all the nerves examined. In particular,
Pathology in peripheral vascular disease nerves

1455

Fig. 5 Transverse sections of the saphenous (A) and sural (B) nerves from Case 10 with chronic PVD showing distinct contrast of myelinated nerve fibre density between the two nerves. The saphenous nerve (A) reveals a complete loss of myelinated nerve fibres, whereas the sural nerve (B) exhibits only moderate reduction in the number of myelinated nerve fibres. Note a myelinated nerve fibre with intramyelinic oedema in the sural nerve (arrow). Plastic-embedded section, thionin and acridine orange staining (A) and methylene blue stain (B). Bars = 30 μm.

Discussion

Pathological nerve changes in acute PVD are dependent on the duration of ischaemia. When pathological changes become apparent, acute PVD nerves showed panfascicular or focal lesions with degeneration of both MFs and UMFs. Major pathological findings in chronic PVD nerves are focal lesions such as selective damage of the central fascicular or subperineurial region, considerable variability between MF densities for individual fascicles of one nerve or individual nerves of one subject, demyelination and remyelination, endoneurial oedema, endothelial swelling and relative preservation of UMFs. Axonal changes were less frequently found in chronic PVD nerves. These pathological changes, except for a high rate of demyelination and remyelination, have been documented in experimental acute ischaemic neuropathy (Nukada and Dyck, 1984; Benstead et al., 1990; Nukada and McMorran, 1994), suggesting that most morphological alterations in chronic PVD nerves may be caused by acute ischaemia/reperfusion.

Experimentally, acute nerve ischaemia induces a localized lesion of axonal degeneration especially of nerve fibres at the central fascicular region ('central fascicular fibre degeneration' or 'ischaemic core') (Korthals and Wisniewski, 1975; Hess et al., 1979; Parry and Brown, 1981; Nukada and Dyck, 1984). In the microembolization model of acute ischaemic neuropathy, an 'ischaemic core' was seen in large fascicles such as sciatic or tibial nerves, but not in peroneal or sural nerves which contain smaller fascicles (Nukada and Dyck, 1984). In PVD nerves an 'ischaemic core' was found also in large fascicles rather than in small fascicles. Central fascicular vulnerability to ischaemia, therefore, could depend on the fascicular size. Localized ischaemic damage at the subperineurial region, contrary to central fascicular fibre degeneration, is described as 'subperineurial fibre
Fig. 6 (A) The sural nerves from Case 13, who had chronic PVD, showing myelinated nerve fibres with disproportionately thin myelin layers relative to axon area. Plastic-embedded 1 μm thick section, thionin and acridine orange staining. Bar = 20 μm. (B and C) Electron micrographs of the peroneal nerve from Case 13 illustrating thinly myelinated nerve fibres (B) and a demyelinated fibre (C). Lead citrate and uranyl acetate staining. Bars = 5 μm.

degeneration' (Nukada and Dyck, 1984; Korthals and Korthals, 1990; Myers et al., 1991; Nukada et al., 1993). These focal lesions cause an increase in variability of MF density which is confirmed by assessing the coefficient of variation and the index of dispersion of MF density. Several weeks after the onset of ischaemia, regenerating nerve fibres become apparent in the localized lesion (Nukada and Dyck, 1986; Korthals and Korthals, 1990). These spatial distributions of acute ischaemic lesions have been described in human vasculitic (Dyck et al., 1972), diabetic (Sugimura and Dyck, 1982; Dyck et al., 1986a, b; Johnson et al., 1986; Said et al., 1994) and chronic multifocal demyelinating neuropathies (Nukada et al., 1989).

If acute ischaemia is implicated in the pathological change of chronic PVD nerves, reperfusion can also play a role in its development. Ischaemic injury could be exaggerated
Pathology in peripheral vascular disease nerves

**Table 4** Frequency of various pathological conditions in single teased fibres from nerves taken from non-diabetic chronic PVD and control (%; mean±SD)

<table>
<thead>
<tr>
<th>Nerves</th>
<th>Normal</th>
<th>Demyelination with or without remyelination*</th>
<th>Remyelination*</th>
<th>Axonal degeneration*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chronic PVD</td>
<td>Controls</td>
<td>Chronic PVD</td>
<td>Controls</td>
</tr>
<tr>
<td>Sural</td>
<td>48.8±6.5†</td>
<td>78.2±10.2</td>
<td>9.2±4.0‡</td>
<td>2.5±3.4</td>
</tr>
<tr>
<td>Saphenous</td>
<td>48.4±5.3†</td>
<td>70.2±4.3</td>
<td>12.2±8.7</td>
<td>3.1±2.8</td>
</tr>
<tr>
<td>Superficial peroneal</td>
<td>51.2±13.5</td>
<td>67.2±9.7</td>
<td>4.2±5.9</td>
<td>7.8±4.0</td>
</tr>
<tr>
<td>Deep peroneal</td>
<td>58.3±10.1†</td>
<td>77.3±10.1</td>
<td>3.4±4.7</td>
<td>1.6±1.4</td>
</tr>
<tr>
<td>Tibial</td>
<td>54.8±6.8†</td>
<td>77.6±3.6</td>
<td>6.9±3.0</td>
<td>3.1±2.7</td>
</tr>
</tbody>
</table>

*Dyck’s grading: C+D = demyelination with/without remyelination; F = remyelination; E+H = axonal degeneration (Dyck et al., 1993); †P < 0.001; ‡0.01 < P < 0.025; ‡‡0.025 < P < 0.05.

*Fig. 7 Transverse sections of the sural nerve from Case 10 showing darkly stained swollen axon (arrow), attenuated or empty axons, and demyelinated nerve fibre. Inset, high magnification of attenuated axon and demyelinated axon from the boxed region. Plastic-embedded 1 urn thick section, methylene blue stain. Bars = 30 μm and 10 μm (inset).*

*Table 5 Density of myelinated nerve fibres in various nerves taken from non-diabetic chronic PVD (number mm\(^{-2}\), mean±SD)*

<table>
<thead>
<tr>
<th>Nerves</th>
<th>Chronic PVD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sural</td>
<td>3550±656*</td>
<td>5316±1598</td>
</tr>
<tr>
<td>Saphenous</td>
<td>2161±1595*</td>
<td>5327±1472</td>
</tr>
<tr>
<td>Superficial peroneal</td>
<td>3254±741†</td>
<td>5233±934</td>
</tr>
<tr>
<td>Deep peroneal</td>
<td>2893±807†</td>
<td>6181±1510</td>
</tr>
<tr>
<td>Tibial</td>
<td>3450±998†</td>
<td>6414±1521</td>
</tr>
</tbody>
</table>

*0.025 < P < 0.05; †0.01 < P < 0.025; ‡P < 0.001.

by subsequent reperfusion, and the peripheral nerve is no exception (Day et al., 1989; Schmelzer et al., 1989). We observed endothelial swelling, endoneurial and intramyelinic oedema and demyelination in ischaemic/reperfused rat hindlimb nerves (Nukada and McMorran, 1994); chronic PVD nerves exhibited these same morphological features. Endoneurial vessels in chronic PVD have been investigated by other investigators showing swollen endothelial cells with ocluded lumen and thickening of basement membrane (Garven et al., 1962; Eames and Lange, 1967). It is of note that some vessels in the present study exhibited reduplication of basement membrane without evidence of diabetes.

Chronic PVD subjects have a history of intermittent claudication and of multiple vascular surgical interventions which could induce acute ischaemic/reperfusion injury to peripheral nerve. Intermittent claudication has been shown to activate neutrophils, increase leucocyte-endothelial adhesion and vascular permeability, and decrease antioxidant levels (Hickey et al., 1993; Edwards et al., 1994; Khaira et al., 1995). Ischaemic/reperfusion injury associated with vascular reconstruction has been evaluated clinically and experimentally, although all the studies have focused on systemic or muscle involvement rather than peripheral nerve (Beyersdorf et al., 1989; Hoch et al., 1991; Adisesiah et al., 1992; Sternbergh and Adelman, 1992). Post-ischaemic rat hindlimb exhibited a transient, early burst of tumour necrosis factor (Sternbergh et al., 1994). Chervu et al. (1989) demonstrated that electrophysiologically, peripheral nerve is more susceptible to ischaemia and short reperfusion intervals than skeletal muscle. In ischaemic limbs with PVD, reperfusion may also occur through collateral channels. Thus, transient ischaemic events including multiple vascular reconstructive interventions may represent a relatively common insult in chronic PVD nerves.

Myelinated fibres with disproportionately thin myelin layers relative to axon area are commonly observed in chronic PVD nerves. Teased nerve fibre study confirmed a significant increase in the number of remyelinated fibres in chronic PVD nerves. A high rate of demyelination and remyelination has been documented previously in PVD nerves (Table 1). Although demyelination has been reported in the models of acute ischaemic neuropathy, such a high frequency of primary demyelination and remyelination has never been induced by an episode of acute nerve ischaemia/reperfusion (Hess et al., 1979; Nukada and Dyck, 1987; Nukada et al., 1993; Nukada and McMorran, 1994). Demyelination in chronic PVD nerves, therefore, may be due to chronic hypoxia. In contrast to acute ischaemic neuropathy, it has been debated whether chronic ischaemia, insufficient to cause infarction, induces pathological changes in peripheral nerve. Demyelinated and
remyelinated nerve fibres have been observed frequently in diabetic subjects whose nerves are chronically hypoxic (Thomas and Lascelles, 1965, 1966; Ohnishi et al., 1983; Newrick et al., 1986; Thomas and Tomlinson, 1993). Systemic hypoxia secondary to chronic obstructive pulmonary disease is associated with neuropathy resulting in slowed nerve conduction and demyelination (Malik et al., 1990). Sladky et al. (1991) developed the model of chronic ischaemic neuropathy using a femoral arterio-venous shunt in rats. After 10 months of 50-70% reduction in nerve blood flow, no evidence of segmental demyelination, axonal degeneration or fibre loss was found in sciatic and tibial nerves. Pathological changes were confined to nodes of Ranvier associated with slowed nerve conduction. Conventional experimental models of chronic ischaemic neuropathy such as galactosaemia, (although the concomitant metabolic derangement may also be injurious), suggested that chronic hypoxia might cause demyelination (Powell and Myers, 1983; Myers and Powell, 1984; Low et al., 1985).

Axonal changes found in chronic PVD nerves, e.g. darkly stained swollen axons and attenuated axons, have been described in acute nerve ischaemia, particularly at the border zone of an 'ischaemic core' (Korthals et al., 1978; Nukada and Dyck, 1984, 1987). Darkly stained swollen axons with disproportionately thin myelin have been reported in superficial peroneal nerves taken from amputated legs in arteriotic diabetic patients (Vital et al., 1983). Axonal changes were more prominent in acute PVD nerves when compared with those in chronic PVD nerves. Experimentally, the severity rather than the duration of ischaemia is more significant in inducing structural changes in peripheral nerve (Sladky et al., 1991; Nukada et al., 1993). Our chronic PVD subjects with a longer history of intermittent claudication did not reveal any different pathology in type and severity when compared with those with a shorter clinical history. Indeed, Eames and Lange (1967) stressed that severity of ischaemia rather than the duration of ischaemia could play a key role in the development of neuropathy in chronic PVD.

What is the underlying mechanism of a complete MF loss in one saphenous nerve? This saphenous nerve revealed extensive degeneration of UMFs, whereas other chronic PVD nerves showed relative preservation of UMFs. Saphenous neuropathy has been described as a complication of vascular reconstructive surgery, possibly due to direct trauma to the nerve during operation (Adar et al., 1979; Røder et al., 1984). The femoral nerve could also be damaged by direct trauma during the insertion of the arterial catheter. Thus, this saphenous nerve lesion is most likely due to direct mechanical injury. An asymptomatic lesion of femoral nerve, concomitant with sciatic neuropathy, has recently been reported as one of complications of vascular surgery (McManis, 1994).

Are there any differences in morphological vulnerability to ischaemia between motor and sensory nerves? This issue, at least in humans, is not resolved. Relative sparing of UMFs in chronic PVD, in contrast to a severe loss of both MFs and UMFs in diabetic PVD nerve, is most likely related to their greater morphological resistance to chronic ischaemia. In the model of acute ischaemic neuropathy, UMFs and small MFs were found to be less vulnerable than large MFs morphologically (Fujimura et al., 1991). Post-ischaemic paraesthesia has been well documented and sensory axons are more prone than motor axons to developing ectopic activity during and after release of nerve ischaemia (Merrington and Nathan, 1949; Poole, 1956). Bostock et al. (1994) has recently confirmed more marked ischaemic susceptibility in sensory axons than in motor axons. A comparative study of motor and sensory nerve conduction in PVD subjects with intermittent claudication showed a significant reduction in amplitude of the sensory action potential in the lateral popliteal nerve, but no significant changes in any other nerve conduction parameters, suggesting that sensory nerve may be more susceptible to ischaemia than motor nerves (Chopra and Hurwitz, 1969a). However, the underlying mechanisms of physiological susceptibility to ischaemia could be different from those of morphological ischaemic vulnerability (Nukada, 1993).

Acknowledgements

This study was supported by the Health Research Council of New Zealand, the New Zealand Lottery Grants Board and the New Zealand Neurological Foundation.

References


Priestley JB. Histopathologic characteristics of peripheral nerves in amputated extremities of patients with arteriosclerosis. J Nerv Ment Dis 1932; 75: 137–43.


Received January 6, 1996. Revised March 15, 1996.
Accepted April 19, 1996.