THE INFLUENCE OF URAEMIC NEUROPATHY ON MUSCLE: EMG, HISTOENZYMATIC AND ULTRASTRUCTURAL CORRELATIONS

G M Savazzi, V Cambi, L Migone, A Marbini*, E Govoni†, M M Bragaglia‡, G Juvarra*, P P Dall’Aglio

Department of Internal Medicine and Nephrology, *Institute of Neurology, University of Parma, †Institute of Electronmicroscopy, University of Bologna, Italy

Haemodialysed patients rarely show signs of neuromuscular damage, and their muscular performance is usually compatible with a normal everyday life. Objective signs are reduction or loss of ankle and knee jerks and muscular wasting.

Muscular enfeeblement can easily be detected by ergometric examinations [1]; the degree of subclinical neuropathy is better indicated by electromyography (EMG).

We have looked into the possible links between nervous and muscular damage by comparing EMG, histochemical and morphological indices.

Materials and methods

Ten patients underwent haemodialysis for a period varying from 24 to 121 months. They complained of easy fatigability and noticeable muscular wasting and these symptoms made us suspect the possible existence of more important muscular damage than in other haemodialysed patients. EMG surveys were carried out using a Medelec MS6 electromyograph, ultrastructural (US) surveys by a Siemens Elmiskop I. Standard methods employed for EMG [2], optical, histochemical and teasing techniques have been described elsewhere [3].

Results

Table I shows the patients’ sex (F,M) and duration of haemodialysis treatment (months) in the first column. The next five columns are divided into two parts and refer to type 1 (1) and to type 2 (2) muscle fibres. Under normal conditions there is a mosaic checkerboard of the different histochemical fibre types; the pathological occurrence of large clusters of one fibre type is named ‘type grouping’ and is associated with collateral sprouting of terminal axons. The presence of type grouping is shown in column 6.

The amplitude (μV) of the motor unit potentials (MUP) is indicated (7th column) as follows: <5000 μV = O, from 5000 to 6000 μV = +, from 6000 to 7000

312
<table>
<thead>
<tr>
<th>Patient</th>
<th>Mean diameter (μ) of muscle fibre type</th>
<th>Atrophy factor</th>
<th>Hypertrophy factor</th>
<th>Subsarcolemmal rims</th>
<th>Type grouping</th>
<th>MUP amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(1)</td>
<td>(2)</td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td>M 75</td>
<td>47.5</td>
<td>23.5</td>
<td>444</td>
<td>920</td>
<td>8.5</td>
<td>0</td>
</tr>
<tr>
<td>M 76</td>
<td>44.4</td>
<td>54.5</td>
<td>388</td>
<td>569</td>
<td>5</td>
<td>210</td>
</tr>
<tr>
<td>M 49</td>
<td>61.5</td>
<td>62.6</td>
<td>24.5</td>
<td>24.8</td>
<td>51</td>
<td>74</td>
</tr>
<tr>
<td>M 89</td>
<td>68.6</td>
<td>62</td>
<td>50</td>
<td>500</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>M 101</td>
<td>47</td>
<td>55</td>
<td>632</td>
<td>420</td>
<td>24</td>
<td>120</td>
</tr>
<tr>
<td>M 100</td>
<td>53.2</td>
<td>53.8</td>
<td>109.4</td>
<td>278</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>F 118</td>
<td>67</td>
<td>56.2</td>
<td>31.3</td>
<td>115</td>
<td>211</td>
<td>69</td>
</tr>
<tr>
<td>F 52</td>
<td>60</td>
<td>39.3</td>
<td>78</td>
<td>787.6</td>
<td>91</td>
<td>0</td>
</tr>
<tr>
<td>F 24</td>
<td>45.5</td>
<td>29.7</td>
<td>477</td>
<td>972</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F 121</td>
<td>54.7</td>
<td>36.2</td>
<td>146.1</td>
<td>1060.3</td>
<td>54</td>
<td>0</td>
</tr>
</tbody>
</table>

Normal values

| M 61 | 65 | 0→150 | 0→150 | 0→300 | 0→500 |
| F 53 | 49 | 0→100 | 0→200 | 0→400 | 0→150 |
\[ \mu V = ++, \text{more than } 7000 = +++ \]. Normal values for reference are also indicated in the Table.

**EMG findings**

A slowing of the maximum motor nerve conduction velocity in the posterior tibial nerve (PTN-MMNCV) and in the peroneal nerve (PN-MMNCV) varying between 27% and 42% was noted, while the distal latency time (DLT) on the same nerves was increased by 23% to 168% (respectively 23%–154% for DLT-PTN and 27%–168% for DLT-PN). The small muscles showed a loss of motor unit potentials (MUP) during maximum voluntary contraction (mixed 46%, minimal pattern 26%) while fibrillation potentials were found in between 25% and 42% of the examined areas. The analysis of MUP indicated the presence of polyphasic potentials from 32% to 75%. MUP of simple form with normal or reduced duration and clearly increased amplitude (\( \geq 5000 \mu V \)) were found in different muscular areas.

**Optic microscopy**

Fascicular biopsy of the sural nerve showed an overall loss of myelin fibres particularly in the axons of the greatest diameter (13–14–15\( \mu \)) so that the normal bimodal appearance of the histograms relating to the cross sectional areas of the fibre population was lost. Teased preparations exhibited figures of paranodal demyelination, coexisting with occasional demyelination of whole internodal segments; remyelination of the demyelinated regions in the form of short myelinated segments was observed. Such pathological findings were found together with intact myelinated fibres.

**Histochemical results**

Atrophy of muscular fibres was seen in all the patients examined: isolated angulated fibres showed loss of reversal between NADH and MenaGPD stains indicating atrophy denervation. Muscular atrophy was easily perceived in type 2 fibres while a less accentuated atrophy of type 1 fibres was indicated by the histograms of the cross-sectional areas. Selective atrophy of the type 2 fibres was found in only one case (patient F 121). Subsarcolemmal rims, intensely stained with NADH were observed especially in type 1 muscle fibres.

**Ultrastructural surveys**

**Sural nerve** In myelinated axons, increase in the ratio of neurofilaments to neurotubules due to abundance of neurofilaments and to relative scarcity of neurotubules was identified, with dilatation and vesiculation of the smooth endoplasmic reticulum profiles and clusters of degenerating mitochondria. Accumulation of lamellated osmiophilic myeloid bodies and/or myelin figures was found. In all cases, limited axonal regeneration explained the sprouting
suggested by the histochemical profile. Lamellar splits and myelin ovoid bodies were suggestive of secondary demyelination phenomena by simple morphology (Figure 1a). More severe US findings consisted in axons extruding from the myelin sheaths to the periphery of the Schwann cells.

![Image](image_url)

**Figure 1a and b.**

*Gastrocnemius muscle* In the uraemic muscle the Z discs of adjacent myofibrils were no longer arranged in register with those of adjoining ones, and some Z discs were streaming while the Z disc material extended into I bands so that the details of the underlying sarcomeres were obscured by the appearance of Z disc granular material. The progression of the alteration was encountered especially in three long term dialysed patients (M 101, F 118, F 121) where a complete disarray of the myofibrillar sarcoplasm was frequently found.

The lack of the normal orientation of the Z lines was associated with an early degeneration and loss of further myofilaments at the periphery of the myofibre and toward its centre; the contractile material was replaced by amorphous granular sarcoplasmic matrix containing particulate glycogen, lipid droplets, lipofuscin granules and mitochondria, Figure 1b. As the myofilamentous damage progressed, the loss of complete myofibrils became more marked as the periphery of the myofibre resulting in the appearance of a subsarcolemmal ring of amorphous sarcoplasmic substance containing only remnants of myofibrils, sarcotubular profiles and mitochondria. In the intermyofibrillar spaces some mitochondria
were of a characteristic hypertrophic size. Initially their growth was perpendicular to the myofibre long axis; later the more hypertrophic mitochondria showed 90° reorientation (longitudinal sentinel mitochondria).

Discussion

Loss of MUP in maximum voluntary contraction tracings and the high percentage of fibrillation potentials present in several muscular areas occur in patients undergoing chronic haemodialysis treatment [4]: these EMG findings indicate the existence of partial denervation. The anatomopathological counterpart of the aforementioned EMG changes can be seen in the peripheral nerve with a decreasing number in all nerve fibre populations. The existence of myelin damage, confirmed by teasing preparations, and axon damage indicated by US, both account for the slowed nerve conduction velocity. DLT was found more compromised than the NCV [5] and this can easily be explained by the fact that DLT selectively investigates the more distal portions of the nerve of the examined limb, compared with the NCV data [6]: this in in keeping with the ‘dying back’ characteristic of uraemic neuropathy [7]. However the slackening of the NCV and the lengthening of the DLT did not always tally and this may be attributed to a lack of synchrony between the degenerative and regenerative phenomena of the nerve fibres.

Apart from uraemia and some experimental neuropathies, various human neuropathies produce secondary muscular damage, mainly but not solely confined to the type 2 muscle fibres. Therefore myopathy in haemodialysed patients can be attributed in most cases to damage to the motor neurone, as the type 2 fibres are considered to be particularly dependent on the state of their own innervation. Atrophy of the type 1 fibres is much less important and could only be perceived from histograms of the cross-sectional areas; some authors have attributed this to loss of tone, disuse and/or direct uraemic intoxication of the fibre. The existence of reparative phenomena in the uraemic nerve was indicated by the appearance, however sporadic, of MUPs well above the normal average amplitude in the small muscles examined.

These potentials are produced by a new pathological innervation of the muscular fibres, arising from the collateral ‘sprouting’ of terminal axons by the motor neurones that have recovered normal vitality and reacquire the ability to re-innervate orphan muscular fibres that had previously been denervated by regression of their own neurones. Histoenzymology, with the discovery of scattered but limited muscular areas of ‘type grouping’ seems to strengthen the above assumptions; the extent of ‘type grouping’ is indirect evidence of the activity of the sprouting phenomena and is an index of the reacquired viability of the motor neurone. In fact in neuropathies which entail loss of nerve fibres but which are on the way to recovery, the re-innervation of denervated adjacent muscle fibres leads to the formation of large clusters of muscle fibres with identical histo-enzymatic make-up (type grouping) indicating active and vigorous sprouting.

These phenomena were very limited in haemodialysed patients and therefore in keeping with MUPs of borderline or wider amplitude than normal in a simple di — or triphasic wave form and of shorter duration, to indicate a well synchro-
nised depolarisation generated by adjacent but limited muscular clusters innervated by the same motor neurone. The significance of such EMG and morphological findings in haemodialysed patients is poor vitality of the motor neurones, as one can see in developing chronic neuropathies, and confirms the high degree of neural damage [8], in spite of the dearth of subjective symptoms. Our results therefore are not in keeping with the current conviction that haemodialytic treatment resolves all the biochemical causes of motor neurone changes, but indicates that uraemic neuropathy usually persists in the haemodialysed patients [8]. In the muscles, ultrastructural preparations indicate a loss of register of the Z discs of adjacent myofibrils, degeneration of myofilaments and their substitution with amorphous granular sarcoplasmic matrix until the loss of complete myofibrils becomes evident; these findings easily account for the muscular wasting, the fatiguability and the loss of strength in haemodialysed patients. Subsarcolemmal rims of hyperplastic mitochondria especially in type I fibres, and 'sentinel' mitochondria in the intermyofibrillar context are ultrastructural findings suggestive of a disadvantageous utilisation of energy.

Two interesting points emerge from the electrophysiological and morphological correlations:

a) neuropathy persists during haemodialysis treatment and gives rise to a considerable degree of secondary myopathy.

b) muscular damage in chronic haemodialysis patients is much greater than that anticipated from the symptoms [3].

References

1. Bundschu HD. Wissenschaftliche Inf Fresenius 1978; 1: 95
7. Savazzi GM. Rivista di Neurobiologia 1978; 7: 13

317